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Behaviour, predator-prey and fisheries interactions of the Ocean sunfish (*Mola mola*) in the north-east Atlantic

by

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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BEHAVIOUR, PREDATOR-PREY AND FISHERIES INTERACTIONS OF THE OCEAN SUNFISH (*MOLA MOLA*) IN THE NORTH-EAST ATLANTIC

Lara Loureiro de Sousa

Over recent years, the availability of satellite telemetry has offered unparalleled opportunities to better understand the behavioural ecology of marine predators. One such predator for which little is known despite the high levels of bycatch in various fishing activities, is the ocean sunfish *Mola mola*. The work presented here tracked sunfish with three different types of satellite transmitter, revealing unknown spatial dynamics and selected habitats for this species in the north-east Atlantic. Tracked fish displayed seasonal movements that were primarily driven by water temperature, while exhibiting pronounced site fidelity to productive frontal regions. Moreover, there was an apparent size-related variation in dispersal, with larger fish moving farther and positively rheotacting in relation to major oceanographic currents. Furthermore, diving behaviour varied both within and between tracked fish, and both reverse and normal DVM were detected. However, as these different diving patterns did not correlate with geographic region or water column stratification, the observed variability is likely driven by prey distribution oscillations. To investigate this further, DNA barcoding of sunfish stomach contents revealed that while the diet of larger fish included cnidarian species, smaller individuals had a more generalist diet. Thus, although no adult sunfish were sampled, these data support the previously reported ontogenetic shift in diet. Additionally, sunfish foraging success was estimated in relation to simulated planktonic prey-fields (e.g. teleost and invertebrate larvae, gelatinous zooplankton). Tracked sunfish performed better than random null tracks in simulated prey-poor areas, whereas they performed equally in more productive regions. Fine-scale GPS tracking of sunfish behaviour also revealed that area restricted search, a proxy for foraging activity, was associated with areas of likely higher productivity. Lastly, coupling the sunfish behavioural and movement patterns, we explored for the first time the extent of sunfish and pelagic longliners co-occurrence. This revealed that 56% of the observed sunfish habitat was concomitantly used by longliners, representing an average of 2 days-at-risk per month – data that can be further used to inform potential management options. Overall, it is presented here the results of an exceptional opportunity to characterise the behavioural ecology, including interactions with prey and fisheries, of the world's heaviest teleost, the *vulnerable* sunfish.

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DECLARATION OF AUTHORSHIP

I, Lara Loureiro de Sousa declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Behaviour, predator-prey and fisheries interactions of the ocean sunfish (*Mola mola*) in the north-east Atlantic

I confirm that:

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To my grandparents, and to Eriana and Letícia!

Definitions and Abbreviations

AIC	Akaike information criterion
ARS	Areas of restricted searching
AUC	Area under the curve
AVHRR	Advanced very high resolution radiometer
AVISO	Archiving, validation and interpretation of satellite oceanographic data
BLAST	Basic local alignment search tool
CCRW	Composite correlated random walk
CNES	French space agency
COI	Cytochrome oxidase subunit I
CPR	Continuous plankton recorder
CPUE	Catch per unit effort
CRW	Correlated random walk
DCRW	First-difference correlated random walk model
DST	Data storage tags
EUMETSAT	European organization of the exploitation of meteorological satellites
FPT	First-passage time
GAMM	Generalised additive mixed model
GMT	Greenwich Mean Time
GoC	Gulf of Cadiz
GOF	Goodness of fit
GPS	Global positioning system

ISRO	Indian space research organization
KDE	Kernel density estimator
KF	Kalman filter
LC	Location class
MCMC	Monte-Carlo Markov Chains
MCP	Minimum convex polygon
MODIS	Moderate resolution imaging spectrometer
MPA	Marine protected areas
NASA	National aeronautics and space administration
nDVM	Normal diel vertical movement
NGS	Next-generation sequencing
NOAA	National oceanic and atmospheric administration
PCR	Polymerase chain reaction
PDT	Profile of depth and temperature
PSAT	Pop-off satellite archival transmitters
PTT	Platform terminal transmitters
rDVM	Reverse diel vertical movement
ROC	Receiver operating characteristic
RSPF	Resource selection probability functions
SIA	Stable isotope analysis
SMW	Split moving window
SOP	Standard operating procedure
SPOT	Smart position only tag

SSH	Sea surface height
SSM	State space modelling
SST	Sea surface temperature
TAD	Time-at-depth
TAT	Time-at-temperature
TL	Total length
UKFsst	Unscented Kalman filter
VMS	Vessel monitoring system
WC-GPE	Wildlife computers global position estimator

Chapter 1: General Introduction

1.1 The ocean sunfish (*Mola mola*, Linnaeus 1758)

Molids (Tetraodontiform - Molidae) include the largest teleost fishes (Johnson and Britz 2005), reaching up to 3m in length and more than 2000 kg in weight (Santini and Tyler 2002). Within the Mola genus, in the Atlantic Ocean, two species are recognized, M. mola (Linnaeus 1758) and M. ramsayi (Giglioli 1883), with the latter being apparently confined to the southern hemisphere (Bass, Dewar et al. 2005).

Molids present several distinct morphological characters, including the clavus, a caudal fin-like structure extending vertically between the posterior ends of the dorsal and anal fins (Johnson and Britz 2005), a degenerate cartilaginous skeleton (Cleland 1862) and the absence of a swim bladder (Watanabe and Sato 2008). This characteristic morphology has stimulated the illustration of these species in the earliest published books on fishes, and the origin of the clavus has been intensively hypothesized (see Johnson and Britz 2005). The vertebral column is severely reduced, with no ribs or pelvic fins resulting in an unbending body, in which both enlarged dorsal and anal fins have become the primary means of locomotion (Watanabe and Sato 2008, see Figure 1 below).

Figure 1 Image of an ocean sunfish *(Mola mola*).

Aiming to understand the mechanisms of sunfish swimming, it was theorised that mechanical strength was of more importance than efficiency, in larger fish (Watanabe and Sato 2008). In this dedicated study the authors found that a subcutaneous layer of low-density gelatinous tissue plays a major role in making ocean sunfish neutrally buoyant (mean body density 1,027 ± 4 kgm-3, N = 20) in seawater (density ca. 1,026 kgm-3) without a swim bladder, which allows the sunfish to frequently descend and ascend in the water column, maintaining their own stroke cycle frequencies. Moreover, despite the increased accumulation of lipids in sunfish liver with growth, it was found that liver represents less than 3% of the total body mass of sunfish. In fact, depending on the body size, at least 69% of the sunfish weight is supported by the gelatinous tissue (Watanabe and Sato 2008). Interestingly, large individuals present higher frequency of fin-beat compared to smaller fish, which contrasts with most fish with a lateral propulsive system where larger individuals show slower fin-beat frequencies (Houghton, Liebsch et al. 2009).

Another distinct aspect of sunfish that has received attention since early descriptions is known as the ‘basking’ behaviour, where sunfish are frequently found just laying at the surface of the water. This behaviour was firstly associated to sick or dying fish (Edwards 2008) but more recently it has been related to either thermoregulation, with sunfish recovering from cold deep waters after extensive dives in the water column (Fulling, Fertl et al. 2007, Potter and Howell 2011, Nakamura, Goto et al. 2015) and/or some form of symbiotic cleaning association with other species (e.g. Laysan albatrosses *Phoebastria immutabilis*) for parasite removal (Abe, Sekiguchi et al. 2012).

Although being very popular worldwide, mainly because of its peculiar shape, descriptive literature on the sunfish, is surprisingly sparse in any area of biology (Watanabe and Sato 2008). Distributed over an extensive geographic area, this epipelagic species appears in warm and temperate zones of all oceans (Pocarsi and Andrews 2001) with accidental captures and/or observation records from various sites in the Mediterranean, North and South Atlantic, Gulf of Mexico and Pacific Ocean (Silvani, Gazo et al. 1999, Sims and Southall 2002, Petersen 2005, Fulling, Fertl et al. 2007, Garibaldi 2015). Notwithstanding, movements, migrations and residence of sunfish are based on few studies (Sims, Queiroz et al. 2009a, Sims, Queiroz et al. 2009b, Dewar, Thys et al. 2010, Potter, Galuardi et al. 2010, Potter and Howell 2011, Thys, Ryan et al. 2015) with few individuals tracked and for shorter periods of time. Hence, in this study, and at the north-east Atlantic scale, three major subjects are focused:

a) Both movements and behaviours of sunfish at different spatial scales are expanded and integrated in the environment;

b) Predator-prey interactions are investigated by firstly empirically detect sunfish dietary habits for the region and then relating the sunfish movements with simulated prey-fields;

c) Lastly, sunfish vulnerability to longliners operating in the region is also estimated.

1.2 Tracking the movement patterns of marine predators

In the marine ecosystem, the aim for a better understanding of individual and population processes led to the development of improved technologies able to record the species locations through time. The tracking of marine organisms evolved to support accurate description of distribution patterns, habitat selection, migrations and foraging strategies, among other ecological factors (e.g. Tremblay, Robinson et al. 2009).

Mark-recapture with coloured wool ribbons was the first tagging technique, employed to document the return of juvenile salmon (*Salmon salar*) to natal rivers in the early 1600s (Kohler and Turner 2001). This procedure involves the capture and the marking of an individual with a noticeable and lasting item before its release back to the environment. Mark-recapture studies provided information on a number of biological and ecological aspects, such as age validation and growth parameters (e.g. Pratt and Casey 1983) or the large-scale migrations of several marine species (Holland, Kajiura et al. 2001, Kohler, Turner et al. 2005, Queiroz, Lima et al. 2005). Certainly, knowledge of marine species distribution, stock structure and exploitation and population dynamics was greatly improved by this tagging technique. However, detailed monitoring of habitat use, behaviours, or the actual route are still hampered with this technique, and the need to recapture the animal, which is largely fishery-dependent (e.g. Queiroz, Lima et al. 2005), often masked the interpretation of the results.

The difficulty of directly observing an individual over extended temporal scales led to the advent of archival data loggers and/or data relay devices (Hooker, Biuw et al. 2007). These animal-attached electronic tags, first acoustically and more recently satellite retrieved, are able to transmit the data without the need to recover the animal, greatly contributing to the understanding of the behavioural ecology of marine species. Various sensors were developed, including swim speed, depth, tail beat frequency and/or heart rate, and the individual’s immediate environment recorders (see Sims 2010). Thus, new information has been gathered on the spatial dynamics of aquatic species, including distribution patterns and habitat selection of highly migratory species (e.g. Priede 1984, Block, Dewar et al. 1998, Block, Dewar et al. 2001, Holland, Kajiura et al. 2001, Stevens, Bradford et al. 2009, Queiroz, Humphries et al. 2010, Stokstad 2005). Moreover, environmental data recorded by these electronic tags, over the last four decades greatly improved the understanding of habitat selection and spatial dynamics of marine species (Evans and Arnold 2009). Ultimately, when coupled with behavioural patterns, the sampling of an animal's immediate environment can be used to forecast possible responses to both environmental and climate changes (Wilson, Grémillet et al. 2002). Importantly, the advent of such technologies have freed the previously dependency on fisheries for tag and data recovery.

Although independent from fisheries and able to provide data on both horizontal and vertical movements (e.g. Carey and Scharold 1990), acoustic telemetric studies are usually time-limited, due to the need to constantly follow the tagged animal (Klimley, Beavers et al. 2002, Klimley, Kihslinger et al. 2005). This led to the advent of tags able to relay stored data, for future satellite transmission of ecological information of an animal activity (e.g. Block, Dewar et al. 2001, Boustany, Davis et al. 2002, Sims, Southall et al. 2003). Thus, the geo-positioning of animal-attached platform terminal transmitters (PTTs) allowed the remote tracking of pelagic species either by using the Argos satellite system or tag-derived light-level data from pop-off satellite archival transmitters (PSAT) (Sims 2010). PSAT tags provide data on diel changes in both thermal preferences and depth profiles and allows the reconstruction of the trajectories made by the animal, through light-level and sea surface temperature-based geolocation (Sims, Southall et al. 2003, Teo, Boustany et al. 2004, Sims, Queiroz et al. 2009). Light-based geolocations are based on the ambient light-level readings with reference to time. Thus, latitude is estimated from either day or night length, and longitude from local midday or midnight time, in relation to Greenwich Mean Time, GMT (Thiebot and Pinaud 2010). However, light levels are affected by several factors such as the orientation of the sensor, the interference of artificial light sources, daily distance travelled, clock drift and/or cloud coverage and the deep diving by the animal (Teo, Boustany et al. 2004, Ekstrom 2007, Thiebot and Pinaud 2010). Also, long-term attachment remains a problem due to either tag failure (e.g. Weng, Castilho et al. 2005) or premature release of the pop-off tag (Lutcavage, Brill et al. 1999, Gunn, Patterson et al. 2003, Arrizabalaga, Pereira et al. 2008), as a result of continuous rubbing of the tether monofilament against the dart, crimps or guillotine, premature corrosion or breakage of the tag's metal pin (Abascal, Mejuto et al. 2010). Furthermore, the accuracy of the estimated locations is less than that obtained from Argos-linked satellite transmitters (Nielsen, Bigelow et al. 2006, Nielsen and Sibert 2007) and, for those species that undertake crepuscular diving at the times when light-level increase (dawn) and decrease (dusk) needs to be measured without animal-induced large amplitude changes, determining movement using light-based geolocation methods is still problematic (Evans, Baer et al. 2011).

The Argos polar-orbiting satellite system has a global coverage and, through direct transmissions, PTT enable geolocations in near-real time, enhancing the understanding of movements of species at a much finer-scale when compared to PSAT technology (e.g. Eckert, Dolar et al. 2002, Weng, Castilho et al. 2005). Notwithstanding, for Argos tags, the time the animal spends at surface is crucial, since the transmitter antenna needs to completely dry for long enough so the tag can transmit recorded data to passing satellites. Hence, the accuracy of Argos satellite transmitters has inherent limitations (e.g. Phillips, Silk et al. 2004), with positions being assumed at the sea-level, with limited ability to estimate velocity and with sometimes infrequent position fixes (MacLean 2009). Moreover, despite the improved resolution in relation to the 100s km associated with PSAT tag geolocations (Sippel, Holdsworth et al. 2011), which greatly informed oceanic movements of different species, Argos is still difficult to resolve behaviours at smaller than the meso-scale ~10 km (Costa, Robinson et al. 2010, Patterson, McConnell et al. 2010).

The Fastloc-GPS^{TM} system, an alternative satellite-linked system with much higher resolution, is becoming more prevalent for bio-tracking research (Tomkiewicz, Fuller et al. 2010). Several studies used this novel approach combining the global positioning system (GPS) location quality estimates and the data-relay capabilities of the Argos system (e.g. Soutullo, Cadahía et al. 2007). Fastloc^{TM} technology allows GPS signals to be acquired by a receiver within less than one second (www.wildtracker.com/fastloc) and position estimates are calculated directly from the data downloaded, instead of on-board the tag, reducing significantly the time required for the GPS antenna to be clear of the water. This rapid acquisition was specifically designed for pelagic animals that surface for only short intervals (Hazel 2009). Current models of tags can store the recorded data for retrieval when the tag is recovered or transmit summaries of GPS data via Argos, as long as the animal surfaces for long enough (Evans and Arnold 2009). Furthermore, tags incorporating Fastloc^{TM} technology may also have sensors to measure depth (from −40 to +1000 m, with 0.5 m resolution), temperature (−40 to +60 °C, with 0.05 °C resolution), light-level (as irradiance at a wavelength of 550nm with a logarithmic range from 5 x 10−12 W.cm−2 to 5 x 10−2 W.cm−2) and to differentiate wet or dry conditions along with archival capabilities, which include at least 64 Mb of memory (see http://www.wildlifecomputers.com/ website for details). These transmitters allow for quick satellite fixes and high accuracy, with at least 60% of the locations within 100 m (Bryant 2007), and were identified as one of the major advances in biologging science (Rutz and Hays 2009), with increasing in scientific use (e.g. Sims, Queiroz et al. 2009). Fine-scale movement data provided by Fastloc-GPS^{TM} instruments are not only valuable for the characterisation of the habitat use, but also for a better understanding on the foraging decisions and population demographics (Kuhn, Tremblay et al. 2010). Ultimately, behaviours of marine predators can now be resolved at the finer scales than previously.

1.3 Horizontal movements

The ecology of marine vertebrates may be revealed by the reconstruction of the animals tracked movements' (e.g. Luschi 2013). For instance, researchers were able to show habitat use was linked to feeding areas in tracked bluefin tuna (*Thunnus thynnus*) (Stokesbury, Teo et al. 2004), whale sharks (*Rhincodon typus*) (Graham, Roberts et al. 2006, Wilson, Polovina et al. 2006), tiger sharks (*Galeocerdo cuvier*) (Fitzpatrick, Thums et al. 2012), southern elephant seals (*Mirounga leonina*) (Bailleul, Charrassin et al. 2007), seabirds (e.g. Jouventin and Weimerskirch 1990, Boersma, Stokes et al. 2002, Shaffer, Tremblay et al. 2006) and sea turtles (e.g. Godley, Richardson et al. 2002, Ceriani, Roth et al. 2012). Likewise, basking sharks (*Cetorhinus maximus)* long-distance routes were found to overlap with biodiversity 'hotspots' along tidal fronts and on shelf-break fronts (Priede 1984, Sims, Southall et al. 2003); and together with genetic analysis, a strong philopatry of white sharks (*Carcharodon carcharias*) in the eastern Pacific to defined oceanic core areas was demonstrated through satellite tracking (Jorgensen, Reeb et al. 2010). Thus, understanding the features that underlie the distribution of oceanic predators has led to a better description of preferred habitats and associated behaviours (Louzao, Pinaud et al. 2011).

Several studies have tracked the horizontal movements of the world's largest (heaviest) teleost, the ocean sunfish (*Mola mola, Linnaeus,* 1758). Acoustic temperature and depth-sensing showed that the sunfish is an active swimmer, moving independently of ocean currents (Cartamil and Lowe 2004), behaviour that was supported by high-accuracy GPS tracking (Sims, Queiroz et al. 2009). In the north-east Atlantic and western Pacific Oceans, a northwards migration in late winter and southward in late summer have been described (Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010). Moreover, the southwards movement prior to or during winter, was also observed in tracked sunfish off the north-west Atlantic (Potter, Galuardi et al. 2010). Being a widespread pattern in ocean migrations and likely driven by plankton productivity (Sims, Queiroz et al. 2009), this seasonal occurrence at higher latitudes has also been shown for other marine species, such as the leatherback turtle (*Dermochelys coriacea*) (McMahon and Hays 2006), the blue shark (*Prionace glauca*) (Queiroz, Lima et al. 2005) and the loggerhead turtle (*Caretta caretta*) (Mansfield, Saba et al. 2009). These species were all found to be associated with sea surface temperature (SST) variations in northern locations. The thermal envelope occupied by the tracked sunfish in the north-east Atlantic, was essentially encompassed by the range 10 – 19°C (> 99% of time) (Sims, Queiroz et al. 2009). In the north-west Atlantic however a different picture was evident with sunfish thermal range being centred on warmer temperatures (17 – 20°C) during the majority of the tracking period (Potter, Galuardi et al. 2010). Water temperatures in the north-east Atlantic, as elsewhere in the world, are changing, with a progressive warming being reported in the last two decades (Hobson, McMahon et al. 2008). Hence, if sunfish are thermally constrained, a northwards expansion together with this seasonal thermal window of favourable conditions is expected (Sims, Queiroz et al. 2009). Tracking horizontal movements of sunfish, for longer periods of time than previously, will provide more detailed information about their movement routes, behaviour and space-use, which, when related to seasonal changes, may elucidate the timing of such movements and possible distribution shifts. In addition, the long-term (> 90 days) GPS tracking of sunfish in the north-east Atlantic, showed intermittent periods of reduced movements in localised areas and faster, directional movements in others (Sims, Queiroz et al. 2009). While the movements of sunfish are presumably linked to restricted thermal tolerances, the authors suggested these pauses to be reflect encounters with patches of preferred prey (Sims, Queiroz et al. 2009, Houghton, Doyle et al., 2006). This was already observed in the filter-feeding basking shark feeding on patchy zooplankton in shelf waters (Sims and Quayle 1998) and in foraging movements of leatherback turtles (Hays, Hobson et al. 2006, Houghton, Doyle et al., 2006). Notwithstanding these recent telemetry studies, the foraging behaviour of sunfish remains poorly understood.

1.4 Vertical movements

Diving behaviours of pelagic species seems to be highly varied. For instance, a study on the diving behaviour of four shark species, the blue, shortfin mako (*Isurus oxyrinchus*), thresher (*Alopias vulpinus*) and bigeye thresher (*Alopias superciliosus*), revealed a clear normal diel behaviour (nDVM), with these species occupying shallower depths at night than during the day, at least for the majority of the tracks (Stevens, Bradford et al. 2009). However, for blue sharks an additional pattern was also found, with less frequent and shallower dives between the surface and depth during the day and less time spent at the surface at night, with no relationship found with either water structure or habitat features. Moreover, blue sharks in the north-east Atlantic also exhibited a preference for surface waters when in stratified water, while irregular behaviours were identified in well-mixed or weakly stratified waters (Queiroz, Humphries et al. 2010). The porbeagle shark (*Lamna nasus*) is also known to move through the water column according to the various characteristics of its thermal structure (Pade, Queiroz et al. 2009); the co-occurrence of the normal diel vertical movement (nDVM) together with reverse diel behaviour (dusk descent–dawn ascent) has been showed in other species such as the basking shark depending on the characteristics of the water column (e.g. frontal versus stratified) and associated with the variable DVM behaviour of zooplankton prey in these different water masses (Sims, Southall et al. 2005). Thus, the structure of the water column may directly, or indirectly, drive prey distribution, and thus altering the distribution of a predator (Queiroz, Humphries et al. 2012). For leatherback turtles, a frequent diving pattern in shallower waters during night and deeper dives around dawn succeeded by preference for surface waters during day has been observed (Hays, Houghton et al. 2004). Turtles were thought to be following the descent of their prey around dawn, until the depth attained is no longer profitable, due to the need to surface for breathing (Hays, Houghton et al. 2004). More recently, while in key foraging areas, turtles appeared to concentrate their foraging effort in the upper part of the water column, spending the majority of time in shallow waters (Fossette, Hobson et al. 2010, Houghton, Doyle et al., 2008). Moreover, deep dives of the whale shark, were found to be influenced by a seasonal predictable food source, with shallower dives made during fish spawning periods (Graham, Roberts et al. 2006). A different study on the humpback whale (*Megaptera novaeangliae*), showed that this species has a pronounced diel difference in depth distribution between surface and bottom feeding behaviour, correlated with vertical changes in the distribution, behaviour and abundance of their main prey, the sand lance (*Ammodytes* spp.) (Friedlaender, Hazen et al. 2009). Taken together, these investigations suggest that the behaviour of a marine predator is likely to change in response to that of the prey.

A normal diel movement pattern has already been described for sunfish, with this species diving below the thermocline during day time, while nocturnal dives were confined to the surface mixed layer and thermocline (Cartamil and Lowe 2004). This classic pattern of nDVM in sunfish was also recorded in the north-east Atlantic (Sims, Queiroz et al. 2009), with individuals spending the majority of their daytime at depth, with vertical movements occurring between dawn and dusk, frequently diving to deeper waters (> 400 m), while resurfacing and using shallower waters during night-time. In the north-east US waters, in which shallower depths prevailed in the summer and early autumn months (often < 10 m) (Potter and Howell 2011). This pattern of preferred surface waters, was suggested to be related to thermoregulation, with the fish being able to re-heat at the surface after deep dives to colder temperatures (e.g. Holland, Brill et al. 1992, Fulling, Fertl et al. 2007). Moreover, it has been suggested that sunfish are unable to spend long periods below 10 ºC, or at least without a prolonged re-warming in surface waters (Sims, Queiroz et al. 2009). This was recently confirmed from sunfish diving profiles recorded off Japan which were directly linked to thermoregulation, with fish being diurnally actively foraging in deep waters, and almost inactive during night-time periods (Nakamura, Goto et al. 2015). Surprisingly, and even though no continuous recorded depth and temperature (time-series) was available for that study, no statistical relationship was obtained between the time spent in cold waters and at surface for sunfish tagged in north-west Atlantic (Potter and Howell 2011). Thus, the vertical movements may well reflect prey searching, as extensive vertical movements might increase the probability of encountering olfactory traces (e.g. Gunn, Stevens et al. 1999), being an improved foraging strategy to the water column structure (Sims, Southall et al. 2008). Thus, analysis of the diving behaviour of marine predators in relation to preferred prey vertical distribution will provide important insights into the fundamental ecology of marine populations.

1.5 Modelling and environmental integration of species behaviours inferred from satellite tracked paths

1.5.1 Track processing and behavioural patterns

Species behavioural and migratory patterns description may be based on the mapping of satellite retrieved positions (Jorgensen, Reeb et al. 2009). However, telemetry has inherent limitations that may hinder the interpretation of such movements and behaviour; data errors are a central problem in modelling animal movements. Furthermore, satellite retrieved data are usually patchy, with temporal gaps of variable length in between individual positions. Thus, different methods have been developed to correct spatial errors and the temporal lags between data points by interpolating between known locations.

The most common approach for the errors' correction is to use the errors in the data to infer the likelihood of a determined position, such as with the so-called state space modelling (SSM) framework. SSMs are valuable procedures, separating the physical process (animal movements) from the observation process (Doppler geolocation of Argos tag positions) (Royer and Lutcavage 2008). As an example, the model developed by Jonsen et al. (2005) accounts for both the estimated error (from inaccurate observations and the process variability from stochasticity in the movement process) and temporal irregularity in the data, and within a correlated random walk (CRW) for location data. Briefly, the model states that the movement follows a Markov chain (MC) and induces correlation in the state of the walk at various stages, with the direction and the distance traversed in one step being governed by a probability distribution (Lal and Bhat 1988). This produces a local directional bias: each step tends to point in the same direction as the previous one, with step orientations being steps uniformly distributed in the long term (Codling, Plank et al. 2008). The CRW is a mathematical method to describe a movement process, in this case correlated, but the random walk is unbiased, without any preferred direction, describing a simply tendency for individuals to persist in their present direction of motion (a localized bias – persistence) (Codling, Plank et al. 2008). The major benefit of this type of modelling is the interpolation into predicted locations that are evenly spaced in time along the track, together with an estimate of confidence, which includes both location accuracy and time between locations (Jonsen, Flemming et al., 2005).

Conversely, Johnson et al., (2008) proposed a continuous time framework of the Kalman filter (KF) (Kalman 1960) to, via maximum likelihood (MLE), estimate parameters and predict unobserved locations along the track. This filtering and smoothing technique is an unbiased estimator for a stochastic linear system (Royer and Lutcavage 2008) and, while minimizing the estimated error covariance (the ultimate goal), is a repetition of the previous *a posteriori* estimates used to project or predict the new *a priori* estimates. Furthermore, the continuous-time modelling permits the use of the data, without subsampling or aggregation, to be treated in a regularly spaced time scale (Johnson, London et al. 2008). Altogether, different techniques have been developed to improve data quality (i.e. location accuracy through likelihood) and thus increase our ability to define better an animal's trajectory and following habitat, distribution and environmental integration inferences.

1.5.2 Environmental integration of movements and behaviour

Habitat selection is a dynamic process during which the animal chooses not only the occupied areas but also the time spent within each of them (Benhamou 2011). Thus, the information on movements and behaviour of animals is best interpreted in the context of its environmental conditions (Weimerskirch 2007). Collecting environmental data, directly from the animals, is of increasing interest to the oceanographic community and one example is the inclusion of these data into both operational and forecasting models of ocean circulation and heat distribution (Fedak 2004). Temperature, water salinity and depth information from bio-logging studies can now be used to characterise the encountered water masses, and to derive measures of water flow and oceanographic activity.

Occupied regions identified from tracking studies can be better characterised from *in situ* sampled environmental data. In fact, cost-effective conservation measures may be facilitated by the identification of consistent ‘hotspots’, areas of elevated abundance (Worm, Sandow et al. 2005), as for instance those revealed by electronic tagging. On the other hand, both the characterisation of areas of high-use and interpretation of animal movements may be assisted by satellite-derived oceanographic data.

Water temperature appears as a major feature influencing the movements of marine vertebrate species, including for instance the bluefin tuna (Lutcavage, Brill et al. 2001), the swordfish (*Xiphias gladius*) (Sedberry and Loefer 2001), blue marlin (*Makaira nigricans*)(Su, Sun et al. 2008), blue shark (Queiroz, Lima et al. 2005), mako shark (Holts and Bedford 1993), salmon shark (*Lamna ditropis*) (Weng, Castilho et al. 2005), loggerhead turtles (Mansfield, Saba et al. 2009) and also ocean sunfish (Sims, Queiroz et al. 2009). Furthermore, presumably due to increased prey availability, it has been linked to the aggregation of marine pelagic vertebrates at shelf beaks, upwelling regions and oceanic fronts (e.g. McCarthy, Heppell et al. 2010). Frontal areas are regions of high primary production, supporting secondary producers and consequently attracting higher trophic levels (e.g. Lutcavage, Brill et al. 2000). Zooplankton and other buoyant organisms are known to passively aggregate at the front, and these accumulations attract higher trophic level predators which forage in these frontal regions (Olson, Hitchcock et al. 1994, Polovina, Kobayashi et al. 2000). This is known to occur with some jellyfish, at least at local scales (Graham, Pagès et al. 2001), bluefin tuna (Lutcavage, Brill et al. 2000, Walli, Teo et al. 2009), swordfish (Seki, Polovina et al. 2002), basking (Sims and Quayle 1998), porbeagle (Pade, Queiroz et al. 2009), blue (Queiroz, Humphries et al. 2010) sharks, both the loggerhead (Polovina, Kobayashi et al. 2000) and the leatherback turtles (Fossette, Hobson et al. 2010), the southern elephant seal (Dragon, Monestiez et al. 2010) and also the ocean sunfish (Sims and Southall 2002, Potter, Galuardi et al. 2010, Thys, Ryan et al. 2015). Moreover, the analysis of animal trajectories along with oceanic currents, estimated from satellite-derived sea level anomaly data, allows the investigation of the environmental integration of the individuals' navigational decisions. Currents have been shown to directly influence the migration routes of adult sea turtles, being crucial to the selection of foraging sites (Hays, Fossette et al. 2010). However, this may imply important adjustments in the migratory patterns, as currents are expected to vary as a consequence of climate change, given the expected change in both ocean and atmosphere circulation by the rising temperatures (Poloczanska, Limpus et al. 2009). Lastly, eddies are known to upwell nutrients into the euphotic zone during their formation and intensification phases (McGillicuddy, Anderson et al. 2007), attracting higher trophic level species. Notwithstanding, as different species have different diet preferences, the abundance of key prey might be more important to foraging success rather than simply elevated levels of overall plankton productivity (Hays, Hobson et al. 2006). While the information on the orientation performance of oceanic migrating species is still scarce, long-term monitoring of sunfish will better inform the factors driving this species' distribution and movements, which in turn may identify sunfish as valuable fine-scale detectors of resource 'hotspots' in the open ocean (Sims, Queiroz et al. 2009).

1.6 Predator-prey interactions

Organisms move through the environment, often with the goal of encountering mates, preferred habitat or prey. Being linked to foraging activities, such as feeding aggregations or areas (e.g. Godley, Richardson et al. 2002, Shaffer, Tremblay et al. 2006) and vertical use of water column (e.g. Sims, Southall et al. 2003, Sims, Southall et al. 2005, Pade, Queiroz et al. 2009, Queiroz, Humphries et al. 2010), the distribution of prey has been suggested to be the main driver of marine predators' movements (Hays, Hobson et al. 2006). Thus, in marine ecosystems predator–prey interactions are fundamental for maintaining the structure of marine communities (Bailey, Ciannelli et al. 2010). However, feeding events may be hindered when a complex prey distribution and stochastic dynamics are coupled in a specific environment. Across the broad range of mesoscale features of the marine environment, the necessary spatial knowledge required for successful foraging will depend largely on the search strategy used (Sims, Southall et al. 2008). Ultimately, encounters of predators and prey will be in proportion to their abundance over space and time. Importantly, the main component of population models that include trophic interactions is the rate of prey consumption by individual predators as a function of prey density (Morales, Moorcroft et al. 2010), but which is poorly known for most marine predators.

In addition, the identification of important foraging or high-use areas is crucial for identifying regions that may warrant special protection status, which ultimately serve as important 'hotspots' for different species. New generation Fastloc-GPS^{TM} tags offer a great opportunity to understand the behavioural processes underpinning the habitat and overall resource use by marine predators. With the high accuracy of GPS technology it is now possible to identify fine-scale behaviours reliably, such as areas of restricted searching (ARS). ARS is characterised by decreased speeds and increasing turning frequency, as an effective response to abundant food resources (Sims and Quayle 1998, Weimerskirch, Pinaud et al. 2007). One measure of time spent between two positions is the first-passage time (FPT) or tortuosity (Fauchald and

Tveraa 2003, Pinaud 2007), and is defined as the time required for a random variable, animal, to cross a circle with a given radius (given starting and endpoint). Generally, calculations of FPT are repeated along the path of the animal by moving the circle at distance *d* and for increasing radii *r* (Bailleul, Lesage et al. 2010). Summing, the circle radius corresponding to the highest variance in FPT is used to define the animal's effective spatial scale (Tremblay, Roberts et al. 2007, Bailleul, Pinaud et al. 2008). Recent studies have been using fine-scale movement and activity data from GPS loggers combined with FPT analysis to examine the spatial scales of foraging and nested search strategy of marine animals, in relation to the physical oceanography (e.g. Northern gannets (*Morus bassanus*) - Hamer, Humphreys et al. 2009). Sims et al. (2009) analysed the changes in movement path tortuosity of sunfish as a function of spatial scale by calculating the FPT. In the latter study, preliminary accounts for 'stop-overs' along the sunfish movements were linked to patch foraging strategies. However, the generality of this behaviour among sunfish has not been determined.

The determination of prey-field distributions and/or abundance in the open-ocean is very difficult. Sunfish is a known planktivorous predator (Dewar, Thys et al. 2010, Pope, Hays et al. 2010, Nakamura, Goto et al. 2015, Thys, Ryan et al. 2015) and although plankton productivity generally increases at higher latitudes in spring and summer (Parsons, Takahashi et al. 1984), the spatio-temporal patterns of zooplankton abundance are fairly poorly described at the scales needed to investigate foraging behaviour in marine predators (Sims, Witt et al. 2006, Houghton, Doyle et al., 2006). Zooplankton are often considered to be inactive organisms, mainly due to their body size and their presumed distribution according to oceanic currents. Ocean currents are known to drive the distribution of organisms with minor swimming capabilities (McManus and Woodson 2012) and thus, zooplankton are likely to be moved and aggregated by these physical processes. Importantly, mesoscale anticyclonic eddies were found to dictate the distribution of plankton species significantly within the Gulf of Alaska for example (Batten and Crawford 2005). In contrast, mesozooplankton such as jellyfish are not distributed inertly (Huse and Fiksen 2010). Vertical movements of jellyfish (*Rhizostoma octopus*) have been investigated using data-loggers and have been shown to undertake extensive vertical movements, with individuals moving up and down through the water column several times per day, rather than just drifting at a fixed depth (Hays, Bastian et al. 2011). Moreover, jellyfish actively swim counter-current, directly responding to current drift (Fossette, Gleiss et al. 2015). Notwithstanding, current advection was found to be an important explanatory factor for the interannual variability in the abundance of gelatinous zooplankton in the northern North Sea (Lynam, Attrill et al. 2009). For smaller zooplankton species (e.g., copepods and molluscs) there is considerable evidence that confirm their lack of ability to swim against horizontal flows, in contrast to the gelatinous species (Fossette, Gleiss et al. 2015). Hence, current-advected productivity may be a good estimation for estimating the prey field of smaller zooplankton, when no *in situ* measurements are available, and may be used to estimate a planktivorous species foraging behavioural patterns and potential encounter success.

1.6.1 Diet of ocean sunfish

Ocean sunfish are often referred to primarily as feeding on gelatinous zooplankton (e.g. Bass, Dewar et al. 2005).There have been several eyewitness accounts of surface predation by sunfish on gelatinous zooplankton despite their relatively small gape which appears less well adapted for capturing and ingesting large scyphozoan jellyfish (Pope, Hays et al. 2010). Nevertheless, sunfish have been associated with jellyfish hotspots (Houghton, Doyle et al. 2006). This study revealed that neither sunfish nor jellyfish randomly distribute themselves, instead they both co-occur more in the same areas than expected by chance. However, the importance of gelatinous prey in the diet of *M. mola* remains uncertain. Interestingly, most fish that eat gelatinous prey are known to have broad diets (Purcell and Arai 2001). Within this context, stable isotope analysis (SIA) has been used to study both diet type and sites along with the trophic position of several different species, being mostly used to infer spatial information of marine mammals and seabirds

(Trueman, MacKenzie et al. 2012). Recent SIA revealed a more broad diet for sunfish of smaller sizes. Juvenile sunfish of the Mediterranean were found to be feeding on a similar proportion of pelagic and neritic prey (Syvaranta, Harrod et al. 2012, Harrod, Syvaranta et al. 2013). More recently, a different study on sunfish off Japan, coupled SIA and swimming stroke efficiency, suggesting that an ontogenic shift in the diet occurred, with smaller individuals probably feeding at the bottom whereas larger sunfish more likely to feed in the water column on gelatinous prey (Nakamura and Sato 2014). Despite these studies, the actual diet of sunfish has not be assessed quantitatively down to the level of individual prey items, hence remains poorly known.

To quantify predator-prey interactions new approaches hold much promise for more detailed studies of ocean sunfish diet. The polymerase chain reaction (PCR) amplification and DNA sequencing of diet contents have been widely applied (Thomas, Jarman et al. 2014). In the marine realm, scat samples are often difficult to obtain, if not impossible for some species, making the stomach contents the only practical methodology, either by flushing or collecting the digestive tracts of dead animals. Items comprising the diet are usually amplified via two different techniques: cloning the amplified DNA or by next-generation sequencing (NGS) technology. Several studies have been successful in assessing diet of marine organisms using cloning, for example in the case of the Steller sea lions (*Eumetopias jubatus* - Deagle, Tollit et al. 2005). In that study, cloning was used to test the efficiency of DNA-based methods in diet reconstructions, and for the Australian sea lion, where 28 prey items were identified using cloning (Peters, Ophelkeller et al. 2014). Furthermore, cloning provides sufficient resolution to detect ontogenetic shifts in diet, for instance in the case of the largemouth bass (*Micropterus salmoides*) from which 26 prey species were retrieved (Jo, Gim et al. 2014). Notwithstanding, NGS has the great advantage of generating thousands of DNA sequences per dietary sample, (De Barba, Miquel et al. 2014). This technology is responsible for the recent increasing number of studies using molecular barcodes to uncover marine species diets, such as those of coral reef fish (Leray, Yang et al. 2013), harbour seals (*Phoca vitulina*) (Deagle, Thomas et al. 2013, De Barba, Miquel et al. 2014), Adélie penguins (*Pygoscelis adeliae*), the endangered pygmy blue whale (*Balaenoptera musculus brevicauda*) (Jarman, Gales et al. 2002) and the little penguin (*Eudyptula minor*) (Deagle, Chiaradia et al. 2010). However, despite the improved ability of DNA analysis to elucidate a species' diet, no studies have hitherto been performed on sunfish from anywhere in the world, to our knowledge.

1.7 Fishing activity and sunfish distribution overlap

Conservation of marine ecosystems requires a better understanding of patterns of biodiversity within preferred areas of fisheries. The increasing interest in the environmental impact of climate change and fisheries on marine ecosystems (e.g. Lynam, Lilley et al. 2011) implies the need for more accurate assessment of population dynamics and individual behaviours. Ultimately, estimated space-use patterns of marine predators will better inform relevant conservation measures. This is more problematic for the majority of pelagic predators which occupy an extensive geographic area and thus, precise information on migration patterns, thermal preferences, behaviour, foraging ecology and residency remains difficult, as described in previous sections.

Being one of the most significant issues affecting fisheries management (Hall, Alverson et al. 2000), incidental captures of non-target species are known to affect both ecosystems and species. Bycatch is the incidental take of undesirable size or age classes of the target species (e.g. juveniles or large females), or the secondary yield of other non-target species. Likewise, undesired individuals caught as bycatch are usually released dead or with injuries (Lewison, Crowder et al. 2004). A study of a longline fishery operating off South Africa estimated 170 sunfish were being captured per 1000 hooks deployed (Petersen 2005). Driven by the high rate of observed incidental captures in fisheries (Silvani, Gazo et al. 1999, Cartamil and Lowe 2004, Tudela, Kai Kai et al. 2005, Fulling, Fertl et al. 2007), there has been an increasing interest in ocean sunfish. In fact, ocean sunfish conservation status was recently updated to *Vulnerable* with the species being facing a high risk of extinction in the wild, based on the assessed levels of exploitation, by the IUCN Red List Categories and Criteria (Jing, Zapfe et al. 2015). Therefore, a better knowledge on the free-living behaviour of this predator is likely to provide information relevant to assessing the potential requirement of bycatch mitigation measures in some fisheries.

Unregulated removal of huge levels of bycatch can result in changes in biodiversity by altering species evenness, whether increasing or decreasing it (Zhou et al. 2010). Furthermore, eliminating predators from ecosystems can trigger trophic cascades that may perpetually modify community structure and disturb the whole ecosystem (e.g. Myers, Baum et al. 2007). Therefore, a more comprehensive understanding of the behaviour and life history traits of a species is required to better assess the ecological impact of their removal from the marine ecosystem (Silvani, Gazo et al. 1999, Cartamil and Lowe 2004, Fulling, Fertl et al. 2007). Whereas currently about 90% of the world's exploited marine fishery resources are controlled by the legislation of coastal states (Garcia and Rosenberg 2010), the remaining 10% are still considered open access resources, presenting problems of regulation, control and enforcement. Increased competition for fish resources implies a global agreement and conventions regarding the use of high seas stocks (e.g. Payne 2000). However, regulations on fishing gear to reduce bycatch together with technological developments to increase the catch of target species, can both have profound impacts on ecosystems (Zhou, Smith et al. 2010). Trophic cascades are expected to occur when predators’ are removed, thus an ecosystem-based approach for marine conservation should comprise the protection of all trophic levels, including predators (Louzao, Pinaud et al. 2011). Importantly, the ecological and habitat preferences of ocean sunfish, including movements and migrations remain largely unknown in many areas where fisheries are particularly intense, in part because of its low commercial value and thus little or no reporting. Sunfish are only known to be a primary catch in eastern Taiwan, where the unit price of meat is ~$9 per kg, with the total annual value of sunfish being about $1 million (Liu, Lee et al. 2009). Hence, despite not being a commercially important species, at present, estimation of ocean sunfish interactions with fisheries is relevant for any future conservation measures that may be necessary, at both species and ecosystems levels.

1.8 Overall objectives

The overall aim of this research, therefore, was to better understand the behaviour and spatial population dynamics of ocean sunfish in relation to its environment at the north-east Atlantic scale. As outlined above, together with the movements of this species at different spatial scales, both predator-prey and fisheries interactions of sunfish in the region are presented. Within this broad framework specific objectives include:

1. Description of the broad-scale movements (horizontal and vertical) of sunfish in the north-east Atlantic.
2. Integration of the movement patterns within the encountered environment through modelling the selected habitat versus the available habitats.
3. Characterisation of predator-prey interactions of the sunfish in relation to simulated fields of plankton informed from empirical abundances.
4. Estimation of sunfish foraging success by relating area restricted searching (ARS) to high-resolution satellite tracking data and with the simulated prey fields.
5. Identification of sunfish diet by means of stomach contents’ DNA amplifications and next generation sequencing techniques.
6. Quantification of the extent of vulnerability of sunfish to surface longliners operating in the north-east Atlantic.
7. To combine the information on spatial dynamics of this species to propose a spatial distributional model for the ocean sunfish in the north Atlantic.

1.9 Thesis synthesis

This thesis follows the preliminary tracking studies of sunfish in the north-east Atlantic (2009), aiming to clarify the movement patterns detected in relation to the environment, at different spatial scales. Thus, different animal-borne tracking systems were used to gather information of sunfish movements and behaviours, which were then integrated with environmental features, simulated prey-fields and fisheries, at different scales. DNA barcoding of sunfish diet emerged to empirically confirm a generalist and likely ontogenetic dietary habits in this species, which conform to both the habitat selection modelled and the behavioural patterns found via tracking. This work built up on one year's *bench fees* provided by a PhD grant from the Foundation for Science and Technology, Portugal, and from private funding of the National Aquarium in Lisbon, Portugal.

The thesis begins with a General Introduction which provides an overview of the current knowledge on ocean sunfish, its value to marine ecosystems and population dynamics and the potential impacts of and upon fisheries. This section highlights the importance of state-of-the-art technologies for the study of marine species´ behaviour and ecology. Also, the overall aims of this study are presented in detail here.

The second section presents a methodological issue regarding different tracking techniques (Argos and GPS). Despite the increased accuracy associated with GPS tags, Argos tracks are still the widest system employed in marine species studies worldwide. In this study, given the simultaneous deployment (of both Argos and GPS technologies - double-tagging of sunfish individuals) a comparison of each tracking system geolocation errors' is performed. Importantly, investigation on two currently employed state space modelling techniques to correct Argos tracks was also made possible. Briefly, it is aimed to understand if less expensive tracking systems can be improved by current available methods to attain such high resolution data. In short, by double tracking ocean sunfish a more detailed answer to the question *Can Argos tracking system be improved to match GPS high accuracy?* is offered.

In the following results chapters it is explored the movements of sunfish at different spatial scales and its integration in the environment. Chapter 3 reveals in an analytical framework the environmental context for the broad-scale movements of sunfish. Satellite tracking of a total of 18 ocean sunfish up to 171 days, since 2007 to present date, enabled both horizontal and vertical migrations to be recorded, which coupled with the remote sensed environmental features informed the habitat selection and environmental cues to which sunfish are responding in the north-east Atlantic. Track-estimated habitat selection was found to be driven by SST and highly influenced by thermal gradients and a distinct displacement and rheotactic behaviour related to sunfish size, which may be linked to an ontogenic shift in sunfish diet. Moreover, four different diving behaviours were recorded, probably associated with prey behavioural differences as no geographical or water column stratification dependencies were found. From previous studies, sunfish was found to have different trophic associations in relation to fish size (Syvaranta, Harrod et al. 2012, Nakamura and Sato 2014) and our results are consistent with divergent prey preferences and feeding strategies.

Afterwards, the predator-prey interactions theme is introduced in the 4th chapter. Here, the fine-scale determination of sunfish dietary habits is revealed through a DNA barcoding approach. In short, sunfish stomach contents were processed and by the combined use of cloning and next-generation sequencing techniques, a total of 41 unique prey items were identified up to the species level. This chapter emerged from the recently advanced possibility of a more generalist predation strategy for sunfish. Typically, generalist predators are key in food webs and their removal is usually related to trophic interaction changes at the community level, with trophic cascades due to strong top-down control elimination (Jennings and Kaiser 1998). Yet, generalist predation is also known to regulate other trophic levels in ways not predicted by cascading trophic interactions (Pace, Cole et al. 1999). Overall, not only the generalist predation was confirmed but also the results in chapter 4 further support an ontogenetic shift in the diet preferences for this species, revealed from previous studies using both video-recording of feeding events and stable isotope analysis.

Knowing both horizontal and vertical movements and behaviour of ocean sunfish together with the information on prey type consumption, a top-down chain interaction may be inspected. In chapter 5 it is explored the foraging success and response to prey for sunfish in the north-east Atlantic. Firstly, the broad-scale movements are inspected in relation to simulated productivity “hotspots” informed by fine-scale currents, remotely sensed. Although, in situ productivity measurements are imperative for this study, the absence of such data drove the estimation of current driven aggregation areas for planktonic species (e.g. gelatinous, teleost and invertebrate larvae stages). Hence, by identifying potential feeding grounds and relating sunfish large-scale movements with these simulated foraging regions, the sunfish predator-prey dynamics are explored in comparison to random (null) tracks. Lastly, the fine-scale (Fastloc-GPS™) movements of sunfish are also investigated and along-the-track areas of restricted search (ARS) are identified. These track sections of increased activity, presumably foraging, in comparison with directed movements were then related to possible explanatory features, in the environment, and to which sunfish are likely responding to. Thus, sunfish ARS was finely described in relation to potential prey “hotspots” driven either by temperature gradients or current shear.

The last results’ chapter reports the fisheries interactions between sunfish and longliners operating in the north-east Atlantic. In light of the previous chapters’ results, the persistence of incidental captures of the cosmopolitan sunfish worldwide (Silvani, Gazo et al. 1999, Cartamil and Lowe 2004, Tudela, Kai Kai et al. 2005, Peterson and McDonell 2007, Pope, Hays et al. 2010, Thys, Ryan et al. 2015) may have important and wider implications for marine ecosystems than previously thought. Although quantifying bycatch is difficult, some studies documented that sunfish totalled impressive percentages of bycatch in different gears (Silvani, Gazo et al. 1999, Peterson and McDonell 2007). Recently, in some regions of the world, this trend is changing suggesting that local population sizes might be decreasing (Peterson and McDonell 2007). This is especially concerning as genetic analyses suggest the existence of several isolated sunfish populations rather than a single global population (Bass, Dewar et al. 2005); further, restricted regional movements have been described for the species (Sims, Queiroz et al. 2009, Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010, Thys, Ryan et al. 2015) and hence this species may be more vulnerable to local depletion than previously thought. Here, the overlap in space and time between satellite tracked sunfish movements and pelagic longliners is analysed.

Finally, the thesis overall results are all discussed in chapter 7. Briefly, a comparison of all different satellite tracking systems is presented, followed by an overview of the three major findings: foraging driven movements, the ontogenetic traits in habitat selection and diet of sunfish, and the conservation assessment. The third section of this General Discussion presents several caveats of this study, suggests future work framework and finalises with a potential distribution of the species, and hypothesises on the spawning region, in the North Atlantic.

Chapter 2: Can Argos be improved to match GPS accuracy? A methodological issue

2.1 Abstract

To better understand the habitat utilisation and behavioural patterns of marine species that surface briefly, the Argos tracking system has been the most extensively technique employed worldwide. Importantly, Argos datasets are still the widest tracking data available despite the technical advance of GPS tracking systems, with spatial errors as small as 60 m. To overcome the uncertainty associated with Argos geolocalisation, state-space models (SSM) have been developed to refine remotely received data. However, quantitative estimations of the errors associated with the SSM interpolated locations in the wild are still deficient. In this study, the accuracy of Argos locations was compared to

GPS positions, acquired from the simultaneous tracking of eight ocean sunfish (*Mola mola)* individuals in the north-east Atlantic. The accuracy of three interpolation techniques (a) linear, (b) Kalman Filter and (c) Markov Chain, was tested. Interestingly, no clear improvement in the errors of processed Argos with the use of either complex SSM was revealed comparing to a simple linear interpolation. Overall average error magnitude was found to be smaller in linear interpolation (2500 m) when compared to both SSM-DCRW (2800 m) and the SSM-KF (3900 m) and significant differences were found for all three interpolation techniques ($H = 13.179$, $df = 2$ and $p = 0.001$). Therefore, and especially for the case of a species that surface only briefly as the sunfish, an overall improvement by applying complex SSM to the remotely retrieved Argos trajectory was not found. Furthermore, the correction of the Argos positions with a Kalman filter, without interpolating the track to a regular temporal interval, only improved significantly poor accuracy locations (LC 0 – B), confirming previous findings. Hence, based on these results, SSM are likely to be highly dependent on the quality of the input dataset, and still fail to reduce the associated error of an Argos good location class. Lastly, despite the efforts of complex frameworks to reduce the inherent inaccuracy of Argos, this system still hampers the detection of fine-scale patterns of marine species. In summary, linear interpolation of satellite tracked sunfish trajectories prevailed as the best approach.

2.2 Background

Used on a wide variety of marine vertebrates, the Argos system has become the predominant platform for remotely collected wildlife telemetry data. However, the system has inherent uncertainty in location precision (Shaffer and Costa 2006). Argos records tag transmissions in modules attached to weather satellites of different Space Agencies (the French Space Agency (CNES), the National Aeronautics and Space Administration (NASA), the National Oceanic and Atmospheric Administration (NOAA), the European Organization of the Exploitation of Meteorological Satellites (EUMETSAT) and the Indian Space Research Organization (ISRO) among others) and download these data back to Earth for processing by Service Argos (Toulouse France, or Landover, U.S.A.). Tag location is calculated from the Doppler shift of the transmitted radio frequency (401.650 MHz ± 30 kHz) and requires a minimum of two successive transmissions during a single satellite pass. Locations are spatially and temporally non-uniformly distributed and subject to varying degrees of positional imprecision determined by the exposure of the tag to passing satellites, the number of transmissions received by the satellite and the stability of the transmitter oscillator (www.Argos-system.org). Official levels of accuracy of location class (LC) are LC3 with a stated error of less than 150 m, LC2 with an error of 150 – 350 m, LC1 with an error of 350 – 1000 m, LC0 with an error greater than 1000 m. LCA and LCB have no error estimates and LCZ implies a failed attempt to obtain a location reported accuracy (CLS 2011). Recently, Fastloc-GPS™, an alternative satellite-linked system with much higher resolution, emerged in marine species' tracking and are becoming very popular in the bio-tracking research community (Tomkiewicz, Fuller et al. 2010). The major benefit of this technology is a truly global coverage, using a constellation of earth-orbiting satellites that continuously transmit information about their location and time (ephemeris and almanac data) to the Earth's surface (Witt, Åkesson et al. 2010). Fastloc-GPS™ tags receive time stamps from 3 or more satellites simultaneously. Subsequently, each time stamp will differ slightly owing to the time taken for the signal from each satellite to be gathered by the tag, and from these differences a location can be triangulated (Breed, Costa et al. 2011). The GPS receivers will either estimate their location and elevation in real-time, or archive the data for later processing of location estimates. Several studies have been using this novel approach combining the GPS quality estimates of location and the data-relay capabilities of Argos System to track organisms' movements (e.g. seabirds, turtles, mammals and teleosts) and investigate possible behaviours (Ryan, Petersen et al. 2004, Schofield, Bishop et al. 2007, Sims, Queiroz et al. 2009, Kuhn, Tremblay et al. 2010, Witt, Åkesson et al. 2010, Votier, Grecian et al. 2011).

The problem of how to best interpret animal locations, given the tracking systems' inherent accuracy errors, is still a recurrent concern in behavioural ecology, and quantitative techniques to process remotely retrieved positions have been developed (Costa, Breed et al. 2012). Over the past decade, great advances have been made in modelling animal movement behaviour. SSM are a relatively new statistical framework that account for stochasticity in the underlying modelled process and errors in the observations, and thus, are generally accepted tools for analysing dynamic processes such as population dynamics and movement (e.g. Patterson, Thomas et al. 2008). These SSMs are time-series methods that allow unobserved states and biological parameters to be estimated from data previously observed with error, and to formulate appropriate movement models by using robust statistical methods (Jonsen, Flemming et al. 2005). Essentially, SSM fit Composite Correlated Random Walk (CCRW) models using movement properties such as turn angles, move lengths, and autocorrelation to infer potential behavioural states from raw telemetry data (Morales, Haydon et al. 2004, Jonsen, Flemming et al. 2005, Johnson, London et al. 2008, Patterson, Basson et al. 2009, Patterson, McConnell et al. 2010, Jonsen, Basson et al. 2013). Two different approaches are often used for analysing tracking data. One is the model proposed by Johnson et al., (2008) based on the Kalman filter to estimate locations at a set of desirable times (SSM-KF). This framework assumes normality of both measurement error and velocity (see Johnson, London et al. 2008 for model details). However, Argos locations have large outliers and are strongly non-Gaussian (Vincent, MCConnell et al. 2002, Jonsen, Flemming et al. 2005), which led to the development of a second approach, the Bayesian state-space model (SSM-DCRWS) proposed by Jonsen et al. (2005), fitted using Markov Chain Monte Carlo (MCMC) instead of KF. The model corrects for the location error and estimates behavioural state, assuming that the two CCRW models, and the respectively described behavioural states (switching state space model), differ only in their values of the mean turn angle (θ) and move persistence (γ) parameters (see more details in Jonsen, Flemming et al. 2005). Given the statistical robustness involving SSM, these frameworks are considered a promising tool for the study of animal behaviour and movements (Patterson, Thomas et al. 2008).

Importantly, the scale of the relationship between habitat covariates and the animal positions is directly influenced by the magnitude of telemetry data location errors (Patterson, McConnell et al. 2010, Montgomery, Roloff et al. 2011). Moreover, habitat data collected via remote sensing or from auxiliary sensors on board the tag are often recorded at times that do not coincide with Argos transmission times. Argos location class attributes have been the subject of substantial investigation (e.g. Le Bouef, Crocker et al. 2000, Costa, Robinson et al. 2010) as this uncertainty in location estimates is crucial for any biological inference resulting from tracking data (Vincent, MCConnell et al. 2002, Bradshaw, Sims et al. 2007, Winship, Jorgensen et al. 2012). Estimation of errors usually involves the placement of transmitters in animals kept temporarily in captivity in a known location for future comparisons of received ones (Vincent, MCConnell et al. 2002); or the application of two different technologies simultaneously on the same animal, one that provides estimates that require validation and one with relatively high precision to which the first can be directly compared (Kuhn, Johnson et al. 2009, Tremblay, Robinson et al. 2009, Winship, Jorgensen et al. 2012).

Several double tracking studies have made use of paired Fastloc-GPS™ Argos linked tags for an estimation of at-sea Argos accuracy, determined as the distance between close-in-time GPS-Argos fixes (Kuhn, Johnson et al. 2009, Costa, Robinson et al. 2010, Patterson, McConnell et al. 2010, Silva, Jonsen et al. 2014, Lowther, Lydersen et al. 2015). In all these cases, Argos datasets were compared to ‘real’ GPS tracks. For instance, the application of CCRW modelling to both GPS and Argos data from northern fur seals (*Callorhinus ursinus*) resulted in Argos locations that were within 5000 m of concurrent GPS location 79% of the time (average location error: 3200 ± 100 and 1700 ± 100 m, respectively) (Kuhn, Johnson et al. 2009). More recently, for five different pinnipeds species (California *Zalophus californianus* and Galapagos *Z.*

wollebaeki sea lions; Cape *Arctocephalus pusillus pusillus* and Australian *A. p. doriferus* fur seals; northern elephant seals *Mirounga angustirostris*) estimated Argos accuracies were generally worse than reported by CLS Argos Service (which up until 2010 were processed using a least squares algorithm – LS), with associated errors varying from 490 m up 10,280 m (68th percentile LC3 and LCB, respectively) (Costa, Robinson et al. 2010). A different study found that Kalman (KF) corrected Argos tracks from two gray seals (*Halichoerus grypus*) were within 8000 m of simultaneous Fastloc-GPS™ positions (Patterson, McConnell et al. 2010). Furthermore, reported error estimates for a newly developed Argos KF algorithm were smaller (5600 ± 5600 m) when compared to LS processed locations (11600 +- 8400 m) (Silva, Jonsen et al. 2014). This algorithm was also applied to Argos data from bearded (*Erignathus barbatus)* and ringed (*Pusa hispida)* seals and resulted in location estimates with accuracies of 4110 m from true GPS positions (Lowther, Lydersen et al. 2015). It is worth noting that while Argos trajectories of marine mammals and birds have been frequently compared to close-in-time GPS fixes, to our knowledge, Argos error estimates are not available for satellite tracked fish species.

In this study, using data retrieved from ocean sunfish (*Mola mola*) tracked with a satellite transmitter equipped with the combination of two tracking systems (Argos and GPS) the actual improvement of retrieved Argos fixes compared to more accurate GPS locations was tested, via implementation of three different interpolation methods. The performance of Argos correction filters (KF) was compared individually per location class.

2.3 Methods

2.3.1 Tagging

A total of eight ocean sunfish were equipped with an integrated Fastloc-GPS™ receiver and Argos Platform Terminal Transmitter (PTT) (Sirtrack Ltd, Havelock North, New Zealand and Wildlife Computers). Sunfish trapped passively in a set-net off southern Portugal were hand caught and tagged underwater. The tags were externally attached to the surface of the fish with either a T-bar anchor tag using a monofilament tether with 1.5 m length or by looping the monofilament in either the base of the dorsal or caudal fins, which was then securely fastened. This tether length was chosen as a trade-off between the need for the tag to have a good chance of breaking the sea surface to transmit in air when the sunfish was near the surface, and to minimise drag and any interference of the tag with fin movements during swimming. Thus, the tag floated clear of the fish, above and behind the dorsum. Battery power was conserved when the tag was submerged via a saltwater switch located near the Argos antenna, with the tag acquiring the GPS position at every 45 s and with subsequent Argos transmission of messages containing the encoded GPS data at every 60 s, when dry in air.

2.3.2 Data acquisition

Two time series of locations were remotely retrieved from towed tags. The first comprised standard Argos locations determined from the Doppler shift in the receiver-uplink signal frequency as the satellite passes overhead. The second was calculated from remotely retrieved GPS data decoded from Argos messages (Wildlife Computers) or by post-processing, using the manufacturer's proprietary software (data available: date, time, latitude, longitude, number of satellites used), respectively.

2.3.3 Data processing

After retrieval of positions via Argos, the recovered dataset was grouped into two subsets for processing according to tracking system. Every GPS position acquired with less than five satellite passes was deleted from the dataset, retaining only locations with reported field-test spatial errors with a of mean 64 m ±79.4 m (Hazel 2009). The resultant GPS tracks were then considered the 'true' path of sunfish and no interpolation was performed. From the Argos dataset, unknown accuracy estimates (LC Z) and erroneous positions (e.g. land) were also ignored (*n* = 3) and remaining positions were further processed using three interpolation techniques.

2.3.4 Statistical framework

For the majority of retrieved locations via each system per individual tag it was evident that locations were not evenly spaced and that the gaps varied. Therefore, errors measured as the distance between concurrent GPS and raw Argos locations (using ArcGIS ESRI) were calculated for different temporal time steps between each tracking-system-derived positions. Here, the maximum temporal gap among each Argos – GPS pair of locations up to which individual behavioural patterns could be discarded from the analysis was explored (non-normally distributed; hence Kruskal-Wallis One Way Analysis of Variance on Ranks was performed in SigmaPlot (Systat Software, San Jose, CA)). Argos errors were estimated as the distance towards a close-in-time GPS position, and resultant estimations between individual tracks were tested for different temporal lags between each pair of retrieved locations [10, 15 and 30 min]. Given the statistical difference in measured distances if pairs of locations were more than 10 min apart among the different individual tracks (Table 2), this temporal lag was selected as the maximum threshold for the two systems to be directly compared. Moreover, both latitude and longitude components of each pair of positions were also compared to investigate possible directional bias in Argos localisation with time.

2.3.4.1 Interpolation of Argos tracks into regular intervals

In this study, three common algorithms were used to interpolate Argos-recovered trajectories into regular time-steps. The resulting fixed trajectories were used to assess the improvement of each method in the accuracy of remotely retrieved positions, by comparison with concurrent GPS paths. To interpolate the Argos tracks, 12 hour time-steps were selected based on the obtained 2.75 fixes per day (average). Thus, the incorporation of at least one real location in the process was ensured.

Firstly, retrieved Argos tracks were linearly interpolated at every 12 hours using MBA Track Analysis software v. 6.39. Secondly for the SSM-DCRW framework, Argos positions were interpolated using the MCMC method in the first difference correlated random walk DCRW model. Here two Monte-Carlo Markov Chains (MCMC) were performed for each model, with 10,000 interactions following a 7,000 burn-in (thin = 5). Briefly, this SSM can be divided in two different parts: the first component of the state–space model is the transition equation, which describes a Markov process where unobservable states evolve over regular time intervals given the previous state, process variability, and biological parameters; and the second component relates the unobserved states predicted by the transition equation to the observed data. We based our models on the code included in the Supplement in Jonsen et al. (2005) and implemented it using the *bsam* package in R software and the Just another Gibbs Sampler (JAGS) 3.2.0 (www.mcmc-jags.sourceforge.net). Furthermore, different time scales were selectively performed to investigate the model performance at different interpolation time steps, and final 12 hour interval tracks were selected for comparison with simultaneous GPS trajectory. Finally for the state-space model using Kalman filter, SSM-KF, the kalman algorithm using the package *crawl* in R was used, also interpolating between the corrected Argos positions at 12 hours. Importantly, input Argos positions were parameterised with the constant (K) error model parameters for longitude and latitude implemented in the *crawl* package (Johnson, London et al. 2008), which are based on the errors estimates in Vincent et al. (2002). Briefly, this SSM consists of two stochastic models, one that describes the movement of an animal (process model) whereas the second describes the actual data with its inherent uncertainty (error) (measurement model), in a state-space version of the continuous-time movement model.

Final interpolation techniques' error estimations were done by calculating the distance between each GPS – interpolated Argos (Linear; SSM-KF or SSM-DCRW) pair of locations retrieved, maintaining the 10 min threshold.

2.3.4.2 Argos individual location class error compared to GPS

Aiming for a detailed analysis per Argos location class, SSM-KF was subsequently run on each track, correcting retrieved locations with inherent error model parameters and without further interpolation. Thus, both corrected SSM-KF and raw Argos tracks were directly compared with a close-in-time GPS location. Finally, descriptive statistics of distance estimations were computed per dataset and non-parametric Mann-Whitney U tests were run to compare between Argos processing.

2.4 Results

From the eight sunfish double tracked in this study (Figure 2) a total of 690 raw Argos (mean 86 ± 63 per track) and 893 GPS (mean 111 ± 64 per track) positions (Table 1) were retrieved. Of these, three sunfish provided more Argos than GPS locations whereas more GPS locations than Argos was the norm, resulting in a mean of 2.75 ± 3.04 Argos and double for GPS (5.33 ± 10.01), per day. Generally, the same overall pattern in movements was detected from both Argos and GPS retrieved positions (Figure 1).

Table 1 Summary of the total eight sunfish double tracked with both Argos and GPS technology

Id	**PTT**	**Date**	**Sunfish length (m)**	**Last Transmission**	**Days liberty**	**No. Argos (avg)**	**avg. Argos d^{-1}**	**No. GPS (avg)**	**avg. GPS d^{-1}**
5	75760	14/05/2008	0.60	29/05/2008	15	23	1.53	46	3.07
6	75761	14/05/2008	0.60	19/05/2008	5	49	9.80	150	30.00
8	75763	06/11/2008	1.00	06/02/2009	92	142	1.54	232	2.52
9	75762	18/05/2010	0.97	06/11/2010	172	125	0.73	206	1.20
10	99102	21/05/2010	1.00	30/05/2010	9	18	2.00	14	1.56
14	15122	18/05/2012	1.00	08/07/2012	51	59	1.16	8	0.16
17	133671	14/10/2013	1.25	26/12/2013	74	77	1.04	116	1.57
18	133672	19/10/2013	1.15	05/12/2013	47	197	4.18	121	2.57

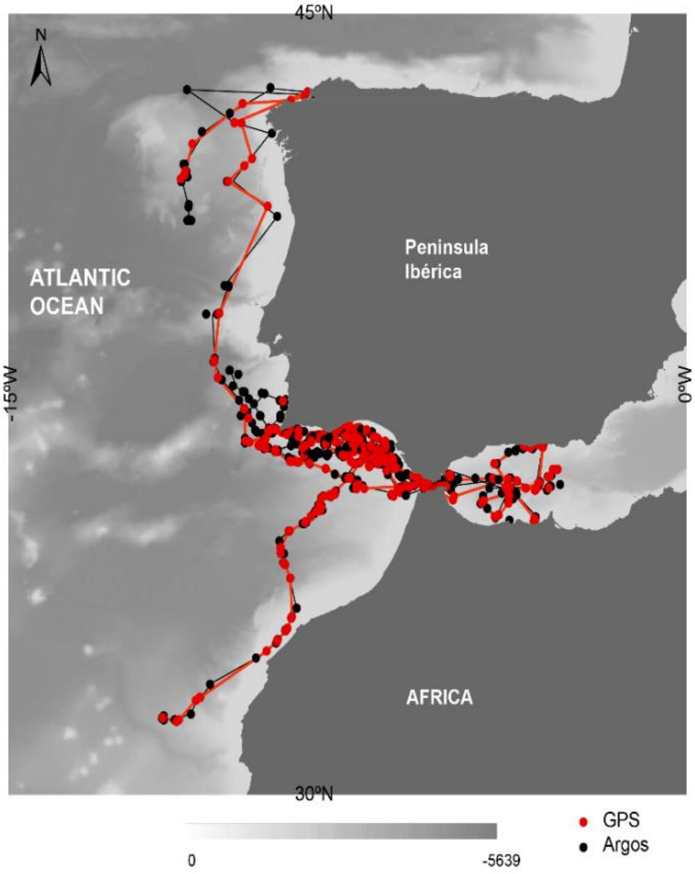

Figure 2 Map of all eight GPS (red) and Argos (black) simultaneously retrieved sunfish tracks analysed. Greyscale bar is water depth in metres.

2.4.1 Overall raw Argos error compared to GPS tracks

With regards to the temporal lag between both datasets, 10 min was found to be the maximum time-step for which no significant difference in individual PTT distances from GPS were found (Table 2), hence pairs of positions separated by less than this time interval were discarded.

A total of 277 pairs of raw Argos – GPS positions were retained within the time-step threshold and, on average, estimated raw Argos error was found to be ~2097 metres (s.d = 7205.7 m). Furthermore, errors in locations estimated from Argos tracks showed the same elliptical distribution in relation to original GPS positions with reference to time, with no significant differences detected between temporal intervals (H = 6.302, df = 5, *p* = 0.278 for longitude and H = 10.174, df = 5, *p* = 0.070 for latitude, Figure 3).

Table 2 Statistical significance (Kruskal-Wallis One Way Analysis of Variance on Ranks) of distances between Argos and GPS per time interval and individual sunfish.

Time Interval	**H**	**df**	***p***
10min	10.318	7	0.171
15min	37.69	7	<0.001
30min	77.38	7	<0.001

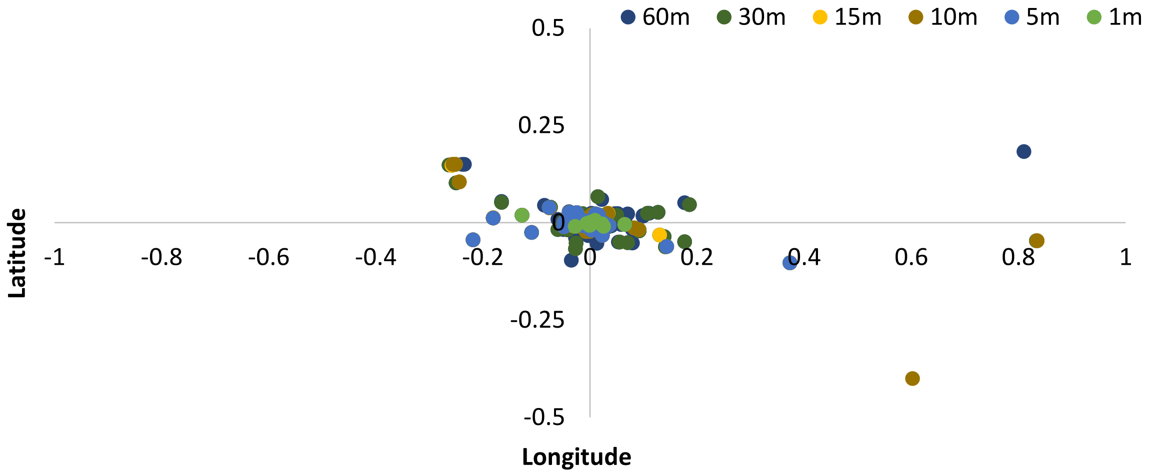

Figure 3 Latitude and longitude components of the errors between Argos and respective GPS positions, according to the time interval analysed. Values are presented in decimal degrees. The similar longitudinal directional bias in the Argos error is highlighted in this plot, independently of temporal interval.

2.4.2 Interpolated Argos error compared to GPS

Retrieved Argos tracks were further interpolated to every 12 hours using all three algorithms and final illustrative maps of each interpolation technique is given in Figure 4. Despite similar visual patterns, the Kruskal – Wallis test revealed that distances towards a close-in-time GPS location to be statistically different between the three methods (*p* = 0.001, H = 13.179, df = 2).

The conservative approach of 12 hours interpolation time-step and the threshold of 10 min between each Argos and GPS positions resulted in a total of 33 concurrent positional pairs retained. Comparing all three methodologies, linear interpolation of the Argos trajectories performed better than the two other complex frameworks, in terms of distance towards an accurate (GPS) sunfish location (Table 3 for descriptive statistics). Errors were found to be on average 2500 m (linear interpolation), 2800 m (SSM-DCRW) and 4000 m (SSM-KF) with similar deviances from the mean for all three methods (4000 – 4500 m).

Table 3 Descriptive statistics comparing the measured distance (in metres) from each Argos interpolation technique-derived position compared to an original GPS location within 10 minutes.

	Linear Interpolation	**SSM-DCRW**	**SSM-KF**
mean	2497.49	2833.58	3938.51
minimum	60.09	99.59	368.21
maximum	16220.30	15456.35	14980.16
variance	20826595.21	18972151.79	15551914.55
standard deviation	4563.62	4355.70	3943.59

Aiming to highlight the importance of time-step choice in the SSM-DCRW framework, different lags in representative SSM-DCRW interpolated tracks from the four longest sunfish tracks (S8-9; S17-18) were tested (Figure 5). Decreasing the time step in SSM-DCRW which, in theory, should diminish the track sinuosity by approximating consecutive positions, forces the model towards greater tortuosity in between retrieved locations (Figure 5). This analysis confirms the variability of the model output depending on the input parameters, especially on the temporal resolution of the raw data.

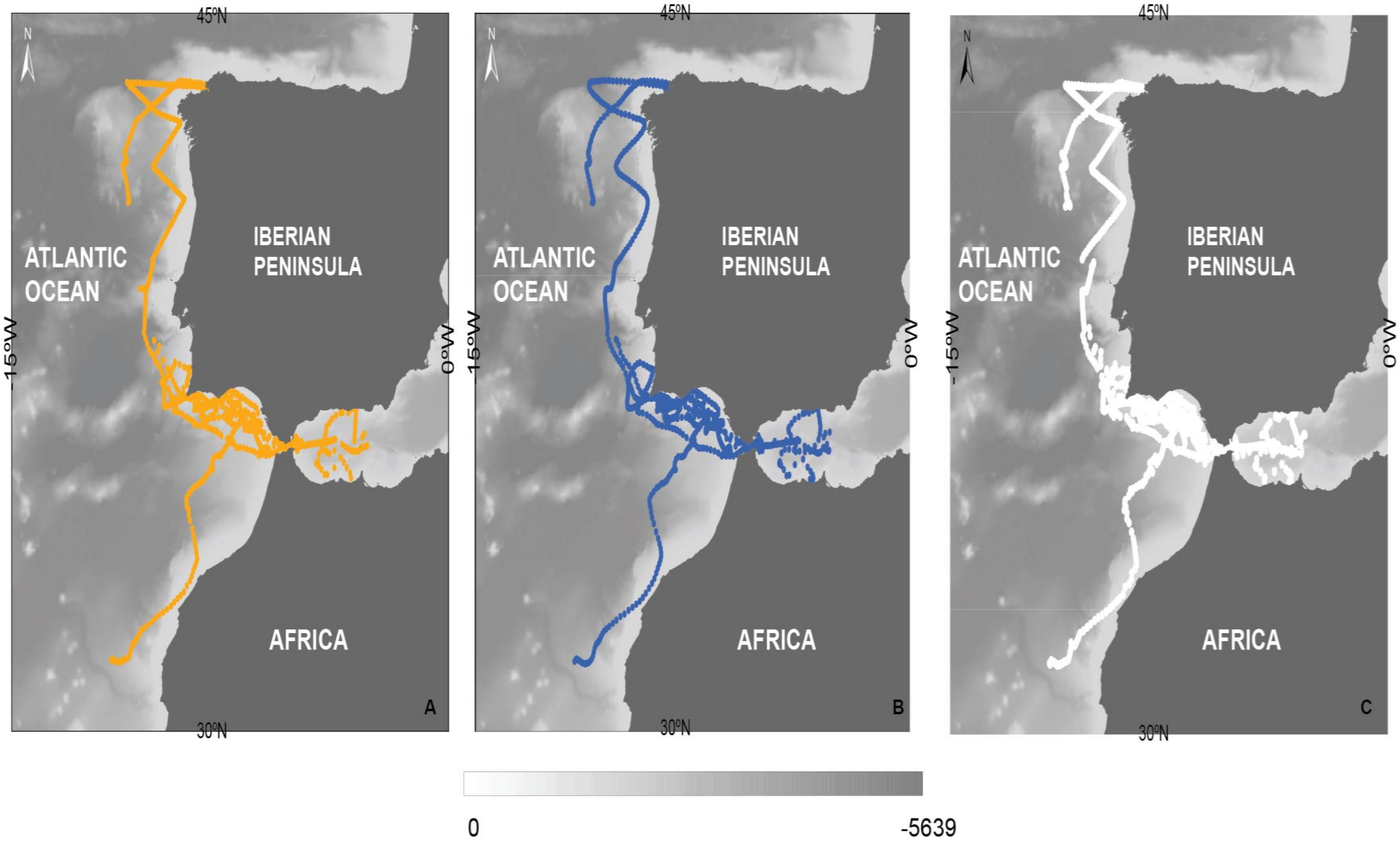

Figure 4 Comparison of all Argos track processing methods: Linear interpolation in yellow (A); SSM-KF in blue (B) and SSM-DCRW in white (C). Greyscale bar is water depth in metres. Note the similarity in the obtained interpolated tracks, at the large scale.

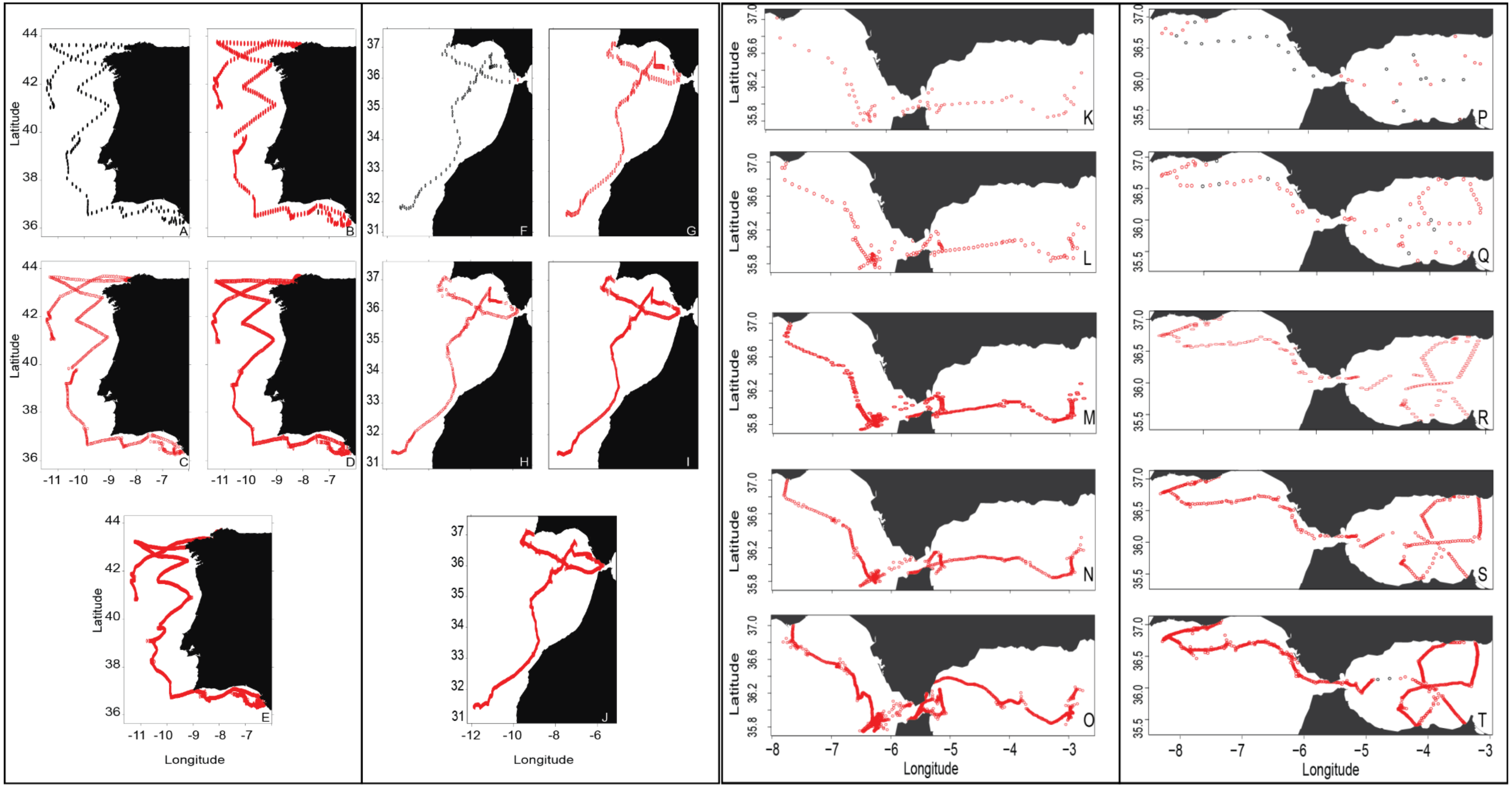

Figure 5 Representative SSM-DCRW model output from four different sunfish tracks (A-E; F-J; K-O and P-T, represent decreasing time steps: 1 day, 12 hours, 6 hours, 3 hours and 1 hour for each individual sunfish respectively). The figures show the SSM-DCRW model forces the path towards greater tortuosity while interpolating the locations retrieved at each time-step interval in order to compensate for the temporal gap in between positions, giving unrealistic tracks.

2.4.3 Per location class – improvement of Kalman filter

Running the SSM-KF to correct only the original Argos positions and discarding interpolated positions allowed us to compare the correction factor at the individual location class (LC) level. Here both raw and SSM-KF Argos tracks in terms of position distance to a close-in-time (10 min) GPS location ($n = 277$) were compared. Positional accuracy regarding the worst LC (0, A and B) was improved when Argos locations are processed using SSM-KF in comparison with the originally retrieved positions. However, this is not detected for the best precision locations (LC 3, 2 and 1 - Table 4). Overall, both datasets (raw and KF – Argos) differed significantly in relation to location distances towards a close-in-time GPS (Mann -Whitney Statistic= 34070 $p = 0.023$).

Briefly, Argos – GPS distances ranged from 27 m (LC3) up to 35,510 m (LCB) if raw Argos tracks were compared, and from 14 m (LC2) to 25,049 m (LCB) in the Kalman filtered tracks. Therefore, these results indicated that the Kalman filter was only effective in reducing the errors associated with Argos locations for positions with lower accuracy.

Importantly, when split per accuracy type (Good – LC 3 to 1; Bad – LC 0 to B), distances towards a close-in-time GPS location significantly decreased in the SSM-KF Argos when compared to the raw Argos tracks, but only at the worst accuracy LCs (Mann-Whitney Statistic = 2143.0 $p < 0.05$). However, comparing both raw and Kalman-filtered Argos, both methods performed similarly in the good accuracy classes (Mann-Whitney Statistic = 18718.0, $p = 0.056$). Detailed boxplots of distances estimated per location class are given in Figure 6.

Table 4 Summary statistics of distances (in metres) between each pair of locations (Argos-GPS) per LC less than 10 min apart. Comparison of GPS with both original and Kalman filtered Argos.

Argos original	**3**	**2**	**1**	**0**	**A**	**B**
Average	326.01	338.43	981.29	2034.37	3477.63	5782.22
Min	27.27	54.80	89.12	333.36	106.41	231.41
Max	1147.02	972.28	20238.43	4491.77	7611.91	35510.90
Percent. 68	378.42	382.85	743.65	2510.67	3979.79	3200.74
Standard deviation	202.78	172.48	2320.13	1405.27	2292.47	8761.12
Variance	41118.73	29750.43	5383002.60	1974778.72	5255433.91	76757154.86

Kalman Argos	3	2	1	0	A	B
Average	325.16	293.35	830.26	1133.79	2929.64	3651.16
Min	64.45	13.72	45.62	383.03	284.29	43.13
Max	967.11	708.24	20238.25	2152.07	7080.94	25049.33
Percent. 68	362.72	368.12	586.69	1264.21	3852.32	2072.71
Standard deviation	184.93	165.25	2333.08	491.65	2291.92	6408.06
Variance	34200.47	26898.90	5371631.12	230207.64	4877673.68	40061689.69
Count	58	67	76	21	14	41

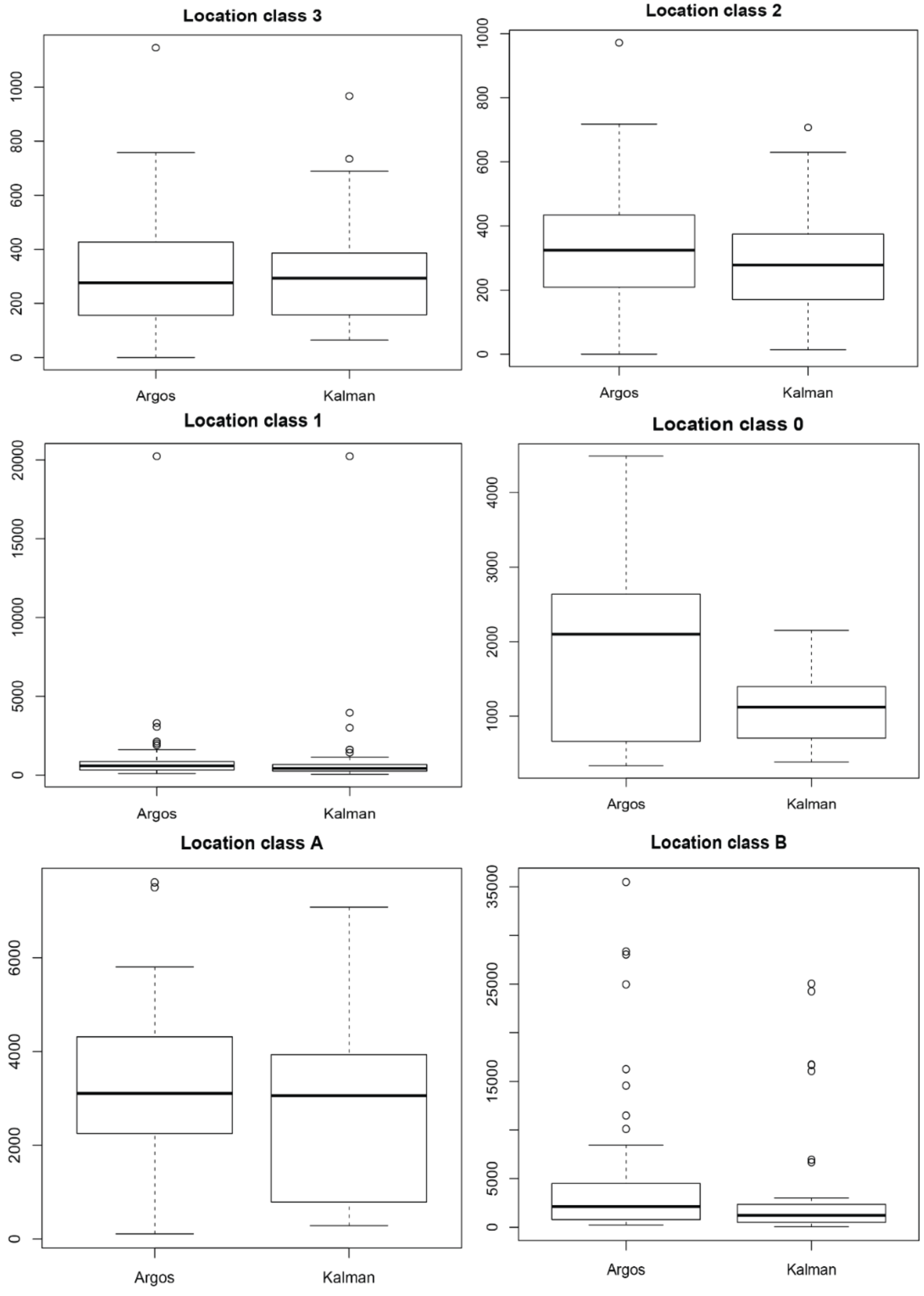

Figure 6 Differences in the measured distance between Argos-GPS pairs per individual location class (3, 2, 1, 0, A and B). Differences only significant at the poor location classes (LC 0 – B), with Kalman reducing the errors acossiated.

2.5 Discussion

This study compared the performance between Argos and GPS tracks, simultaneously retrieved from eight double-tagged sunfish individuals, at sea. Overall, an estimated error of 2100 m for Argos received tracks was found on average, when compared to close-in-time GPS positions (≤ 10 minutes) and no differences (biases) were found in either latitude or longitude components of estimated errors. The double-tag dataset allowed us to further investigate the performance of three different interpolation techniques to Argos tracks and quantify the improvement of each methodology for Argos positional accuracy in relation to the GPS data. Taken together, linear interpolated Argos locations were, significantly closer to a GPS location (2497.49m) than SSM-DCRW (2833.58 m) or SSM-KF (3938.5 m) positions. Therefore, at least for sunfish tracked in the north-east Atlantic, and in an overall movement analysis, using either of the SSM techniques did not improve significantly the accuracy of Argos retrieved locations. Lastly, and in a more detailed analysis (per location class), correcting Argos with SSM-KF only improves the accuracy of the original worst location class positions (0-B). Ultimately, this study contributes further to empirical estimates of Argos system precision from tracked animals in the wild, aiding future animal track reconstruction issues. Importantly, this is, to our knowledge, the first Argos-GPS error comparison made for a teleost fish species at sea.

2.5.1 Importance of tracking system improved accuracies

In this study, similar general, broad-scale patterns of sunfish movements and distribution in the region were evident without any analysis of both tracking systems' retrieved positions. This finding confirms the utility and relative accuracy of Argos geolocations for marine species broad-scale studies (Costa, Robinson et al. 2010). This is of more importance given the fact that Argos is the most frequently employed system in marine tracking studies (Shaffer and Costa 2006). Yet, within the marine ecosystem improved location accuracy estimation from biologging devices is essential to further investigate biological and ecological aspects of species. For instance, a behaviour such as foraging (that includes fine-scale path tortuosity during intense searching) is frequently identified by path sinuosity, increased turning angles and decreased speed (Fauchald and Tveraa 2003, Patterson, Thomas et al. 2008, Dragon, Bar-Hen et al. 2012). All these parameters are derived from location estimations, frequently with diminished accuracy. Ultimately, improved quality tracking data will better inform habitat use patterns, which linked to environmental data will further sustain both individual, populations and species level habitat preferences (Block, Jonsen et al. 2011). Thus, scrutiny of current tracking system's accuracy potentially improves the knowledge of marine species distribution and behaviours, helping to inform possible management and conservation measures.

Estimation of the accuracy of different tracking systems requires double-tracking experiments in free ranging animals at sea (Winship, Jorgensen et al. 2012). In this study, combining GPS raw data and both linear and more complex SSM of Argos retrieved locations allowed us to estimate both directly retrieved and interpolated location errors from Argos-tracked sunfish. Frequently, from double-tracking experiments, GPS locations are interpolated to match the precise timing of Argos positions retrieval and, even though the estimated error of Fastloc – GPS is 10 to 40 times smaller than Argos inherent accuracy (Dujon, Lindstrom et al. 2014), the analyses do not account for the inherent interpolation errors, which are not estimated for the GPS path. Therefore, to avoid introducing extra bias in the estimations (Witt, Åkesson et al. 2010), and despite the fact that both Argos and Fastloc-GPS™ locations were not precisely simultaneously collected within an individual, the Fastloc-GPS^{TM} derived track was not interpolated to evaluate the location class of Argos-derived locations. Therefore, in a more conservative approach GPS processed positions were assumed to be the most accurate ones and to which both Argos raw and corrected/interpolated locations were directly compared.

2.5.2 Argos vs. GPS: important findings

We found that individual tracking datasets have different error magnitudes (distances between each Argos-GPS pair) if pairs are more than 10 min apart in time, confirming the distinct diffusivity inherent to each tracked individual (Jonsen, Basson et al. 2013). This variability in the errors among individual tracks has been previously noted where both magnitude and directionality of light-level (PSAT) spatial accuracy varied among individual tracks (Winship, Jorgensen et al. 2012); or the exacerbation of the differences in accuracy among different PTTs by the application of filters such as the Kalman filter applied by the Argos system (Boyd and Brightsmith 2013). However, in recent similar studies, possible individual differences were not accounted for or were only considered in a hierarchical modelling approach (Silva, Jonsen et al. 2014, Lowther, Lydersen et al. 2015). Here, with the conservative approach of considering only pairs within 10 min of each other, no individual behavioural bias, detected for longer time-steps, was introduced in the analysis. Interestingly, both latitude and longitude error components revealed no differences when comparing different temporal intervals, with the same structure of a longitudinal directional bias being maintained. This result conforms to previous findings (e.g. Silva, Jonsen et al. 2014) and were not included in the error estimation in this study following the recent findings of the lack of correspondence between the magnitudes of each component axis and the actual spatial errors (Boyd and Brightsmith 2013).

In wildlife telemetry studies, improper data treatment is likely to either produce erroneous results or mask important ecological patterns or behaviours (Bradshaw, Sims et al. 2007, Sims, Righton et al. 2007). Therefore, different frameworks have evolved in terms of reconstructing the most probable track of free-ranging animals, addressing the spatial error of the retrieved locations, for example, the well-known and widely applied SSM (Jonsen, Myers et al. 2007, Bailey, Shillinger et al. 2008, Johnson, London et al. 2008, Patterson, Thomas et al. 2008, Flemming, Jonsen et al. 2010, Lopez and Malardé 2011, Sippel, Holdsworth et al. 2011, Boyd and Brightsmith 2013, Jonsen, Basson et al. 2013, Silva, Jonsen et al. 2014). Yet, these results suggest that classic linear interpolation applied to raw Argos tracks appears robust in temporally regularising the remotely gathered free-ranging ocean sunfish Argos time series, outperforming both the other two complex SSM methods. Furthermore, both SSMs employed in our analysis depend largely on the results of Vincent, et al. (2002), as both techniques are based on the empirically-derived measurements by the latter study. Several studies suggested further calibrations to improve these estimations (Jonsen, Flemming et al. 2005, Johnson, London et al. 2008, Patterson, McConnell et al. 2010, Jonsen, Basson et al. 2013). Notwithstanding, without a complete understanding of the track parameters, either the accuracy or the positions' frequency, it is possible that the behavioural states may well be the location estimates' noise (Costa, Robinson et al. 2010). Importantly, regarding SSM-DCRW the analysis confirmed the model dependency on the input data. Briefly, large data gaps require greater care when movement behaviour is to be inferred (Jonsen, Basson et al. 2013), and the current available methods still fail in explicitly model the time between locations, even if a regularisation step is performed (Jonsen, Flemming et al. 2005). Thus, despite the widely accepted enhanced value of SSM to improve statistical analysis of animal behavioural data (Jonsen, Flemming et al. 2005, Block, Jonsen et al. 2011, Winship, Jorgensen et al. 2012, Silva, Jonsen et al. 2014), the technique is still not able to model movement characteristics in between retrieved positions, especially when the time gap widens, as is often the case for fish that do not need to surface to breathe air. Importantly, results for ocean sunfish do not conform to the widely accepted result showing good improvements made to retrieved Argos tracks by employing SSM.

Continuous modelling and interpolation of Argos retrieved tracks, although proven valuable and necessary for several analysis to be performed, such as habitat use (Tremblay, Shaffer et al. 2006), may imply the exclusion of a substantial proportion of the raw dataset (Patterson, McConnell et al. 2010). This disadvantage is enlarged in species that surface only briefly, with decreased number of available positions. In this case, discarding data is likely to be more problematic. For a second approach, the CRW Kalman smoothing corrected the raw Argos locations without interpolating the dataset. Distance of each Argos location towards a close-in-time GPS position was measured (threshold of 10 min providing no individual bias is added). This framework enabled the analysis of the errors associated with each of the known Argos LC individually and these to be compared to the highly accurate GPS tracking system. Interestingly, although significant differences were found among the two datasets (raw and SSM-KF processed Argos), these were only present in poor location accuracy classes (LC 0 – B). A very recent study, reporting errors of the new model algorithm provided by Argos system, the Square Root Unscented Kalman Filter (SRUKF www.Argos-system.org), compared to Fastloc GPS™, also determined an increase in the accuracy of LCs of poor accuracy (0 – B), but not on the best quality (3 – 1) ones (Lowther, Lydersen et al. 2015). Following these results, our findings also provide a better accuracy for lower quality LCs, reflecting the dependency of SSM on the input quality data. Surprisingly, the magnitude of KF processed least square Argos location errors found in this study is consistent with this recently reported SSM – SRUKF Argos locations, from two air-breather marine animals, the bearded and ringed seals (*Erignathus barbatus* and *Pusa hispida*, Lowther, Lydersen et al. 2015). The fact that the majority of retrieved Argos positions of sunfish were of good accuracy (LC 3 – 1) may explain the lack of an overall improvement of Argos tracking data in this study by both SSM techniques. This follows Silva et al., (2014), that an overall average error of Argos modelled tracks is increased with the number of poor location classes. The findings of the latter study confirm the increased accuracy of Argos when KF is implemented, but also raise numerous concerns with regards to raw data quality input (Silva, Jonsen et al. 2014). Briefly, tracks composed by larger distances between consecutive locations and/or increased number of poor location classes (0 – B) had increased overall associated error (Silva, Jonsen et al. 2014). In the present study, input data quality was not as problematic, with more than 70% of the locations being of classes 3 – 1. Altogether, our results conform to previous findings of a good improvement of SSM-KF in lower accuracy Argos locations, however for the higher accuracy locations defined by Argos, no difference is found between original and SSM Argos.

In addition, smaller Argos errors than the majority of studies comparing both tracking systems are reported (e.g. Hazel 2009, Costa, Robinson et al. 2010, Patterson, McConnell et al. 2010, Hoenner, Whiting et al. 2012, Silva, Jonsen et al. 2014, Lowther, Lydersen et al. 2015 and the studies referenced within). Interestingly, despite the lack of surface times obligatory dependency, satellite retrieved tracks from sunfish are of better accuracy than those collected from marine mammals, even if increased number of locations were retrieved (e.g. 1 per hour Lowther, Lydersen et al. 2015). Notwithstanding, marine wildlife telemetry data generally consist of a majority of poor accuracy LC, either driven by individual behaviours or by the influence of the occupied environment (e.g. Hays, Åkeson et al. 2001, Hammerschlag, Gallagher et al. 2011) which emphasises the need of increased accuracy in the techniques used by the Argos system for location processing. Therefore, present results are in line with the concern that raw data quality input is of great importance in these comparative studies and caution should be taken when applying either interpolation method. It was shown here that for some species, such as the sunfish, the SSM frameworks do not improve the overall retrieved Argos accuracy. Moreover, SSM – KF is only effective in lower accuracy locations. Importantly, both avian and marine mammals' tracks are often analysed to investigate Argos system errors and, to our knowledge, no dedicated study has assessed the accuracy of fish retrieved Argos locations compared to simultaneous GPS localisations. Surprisingly, despite the lack of continuous or frequent surface times for breathing, sunfish retrieved positions were good enough for a simple linear interpolation to be applied, without the cost of increased associated errors.

2.5.3 Implications in future studies

The higher-accuracy of GPS tracks and the increased use of such technology are likely to be responsible for a much more detailed analysis of the spatiotemporal patterns of animals' trajectories and behaviours in the future (Schofield, Bishop et al. 2007, Hazel 2009, Hays, Mortimer et al. 2014). In fact, our findings confirm the increased spatial errors associated with the Argos system, i.e. that track data post-processed with SSM fail to provide finer resolution (below the 1000s of metres accuracy) for reliable identification of potential behaviours. Assessments of such detail will certainly contribute to a better knowledge of long-distance migrations, home-ranges, influence or impacts of ocean currents, swimming mechanisms and, ultimately, the environmental drivers for a species' migration. However, determining the distribution of Fastloc-GPS^{TM} errors is still lacking and is currently needed (Winship, Jorgensen et al. 2012) if the aim is to improve the scope of telemetry spatial accuracy analysis. Furthermore, Argos tags remain the widest source of data on pelagic species, which increases the necessity of improving this type of data quality (Douglas, Weinzierl et al. 2012). Caution is then needed when interpreting animal movement patterns and associated behavioural states based on SSM processed Argos tracks. Future work should investigate the impact of track quality parameters, such as accuracy and frequency of positions on biological interpretation of animal movement data. Moreover, despite the robust patterns presented here, the results rely on a small number of tracked individuals, thus, larger sample size together with longer deployments would likely improve the outcome of a double tracking experiment.

Chapter 3: Environmental influence on the satellite tracked movements of sunfish

3.1 Abstract

Determining the habitat use of mobile marine species is important for understanding responses to climate change and aids the implementation of management and conservation measures. Inference of preferred habitat use has been greatly improved by combining satellite-based oceanographic data with animal tracking techniques. Although there have been several satellite-tracking studies on ocean sunfish, limited information is available about either horizontal or vertical environmental preferences. In this study, geographical movements and diving behaviour of ocean sunfish (*Mola mola*) were explored together with the environmental factors influencing this species' space use in the north-east Atlantic. Satellite tracking (up to 171 days, mean 66 days) revealed seasonal movements, with larger individuals (> 0.92 m TL) travelling further and diving deeper than smaller fish. Significant variations in the trajectories of tagged sunfish were recorded, intrinsically linked to the size-dependent rheotaxis. Sunfish spent extended periods in three focal areas, the Gulf of Cadiz, north-east Iberia and the Alboran gyre, which are characterised by the presence of frontal features with elevated primary production. Habitat modelling revealed that sea surface temperature, thermal gradients and chlorophyll *a* significantly influenced sunfish distribution. Diving profiles, extending from the surface to a maximum depth of 704 m, revealed different depth use patterns not linked to geographic region or water column stratification, but possibly related to prey distribution. These observations of considerable variability in diving patterns over short time periods likely reflect the tracking of planktonic prey vertical movements. Lastly, smaller sunfish swam less frequently against major currents with movements constrained nearer to the tagging area in in contrast to larger individuals. Therefore, the present findings suggest a size-dependent shift in movement strategies, which is in line with the recently described ontogenic change in the diet for this species. Taken together, sunfish movements and behaviours are likely linked to differences in diet preference and subsequent energetic requirements.

3.2 Background

Knowledge of seasonal movements and migratory routes is crucial for understanding the distribution of animal populations and represents a first step in the investigation of the ecological mechanisms underlying spatial dynamics (Weng, Foley et al. 2008, Chapman, Hulthén et al. 2012). In marine animals that spend short periods of time at the water's surface, such as pelagic fish, the understanding of seasonal movements is complicated by the difficulty of making sustained direct observations over the larger spatial scales relevant to migration (Gurarie, Andrews et al. 2009). However, in recent years, techniques such as electronic tagging have been developed to track marine species' individual movements (Humphries, Queiroz et al. 2010, Sims 2010, Block, Jonsen et al. 2011) by deploying diverse tag types (Walli, Teo et al. 2009, Queiroz, Humphries et al. 2010, Block, Jonsen et al. 2011). Coupled with remote sensing of environmental variables, satellite tracking has improved our knowledge of habitat selection patterns and fluctuations in environmental preferences (Shepard, Ahmed et al. 2006). While movements are a measurable behavioural response to a combination of factors including internal states, physiological constraints and environmental variations (Gurarie, Andrews et al. 2009), the description of important habitats relies on identifying the features underpinning species distributions (Louzao, Pinaud et al. 2011).

A number of environmental variables have been linked to marine species distributions. For instance, water temperature has been described as a major environmental driver of the movements of diverse marine species including bluefin tuna (*Thunnus thynnus*, Lutcavage, Brill et al. 1999), swordfish (Sedberry and Loefer 2001), blue shark (*Blue shark,* Queiroz, Humphries et al. 2010), mako shark (*Isurus oxyrinchus,* Holts and Bedford 1993), salmon sharks (*Lamna ditropis*, Weng, Foley et al. 2008) and loggerhead turtles (*Caretta caretta,* Mansfield, Saba et al. 2009). The aggregation of marine pelagic vertebrates in areas dominated by steep thermal gradients such as tidal and shelf break fronts, upwelling regions, mesoscale eddies and oceanic fronts has also been documented widely (for review see Scales, Miller et al. 2014). Chlorophyll *a* was also found to influence the distribution and migration of loggerhead turtles, albacore tuna (*Thunnus alalunga*) (Polovina, Howell et al. 2001) and whale sharks (McKinney, Hoffmayer et al. 2012). Hence, the importance of these oceanographic features is likely due to improved foraging opportunities (Hays, Hobson et al. 2006).

Geostrophic currents are another important feature likely shaping habitat selection and animals' distribution, where rheotaxis (body positioning in relation to the direction of the flow) is a multisensory behaviour found in many aquatic organisms (Bak-Coleman, Butail et al. 2012). Currents sway the directional performances underlying the movements of species and at a broader scale, the migrations of a population (Luschi 2013). Rheotaxis can either be positive (body against the flow) or negative (going with the currents' direction) (Kobayashi, Polovina et al. 2008). Different studies have focused on the influence of ocean currents on recorded marine species trajectories (e.g. Polovina, Kobayashi et al. 2000, Luschi, Sale et al. 2003, Gaspar, Georges et al. 2006), revealing the importance of such features in along-path decisions. One of the best known examples of the currents influencing an organism movements is the European eel (*Anguilla anguilla*) migration to the Sargasso Sea (6000 km) which is believed to be aided by eastern boundary currents (van den Thillart, Palstra et al. 2009). In fact, surface currents entail a strong but highly variable impact on the movements of marine animals, as demonstrated for leatherback turtles, where currents were found to distort the animal path (Gaspar, Georges et al. 2006). More recently, juvenile loggerhead turtles were found to detect the current flow, responding positively to its direction (Kobayashi, Polovina et al. 2008). No similar studies have been performed for sunfish yet, with no relation between sunfish movements and the currents directionality being tested.

Several analytical techniques have been developed in the past few decades to model species distributions, from traditional null hypothesis testing to a more complete framework to test competing hypotheses (see review in Johnson and Omland 2004). Current species distribution models usually relate field observations to environmental predictors, based on statistically or theoretically derived 'surfaces' of species response to the habitats (Guisan and Zimmermann 2000). From regression-based techniques, such as generalised linear and additive models, to more sophisticated machine learning informative models (Olden, Lawler et al. 2008), the basic concept of combining observations of species occurrence or abundance with environmental estimates has been widely explored (Guisan, Edwards Jr. et al. 2002, Guisan, Graham et al. 2007, Elith and Leathwick 2009, Abecassis, Dewar et al. 2012). Non-linear response, occurrence or abundance of species can now be modelled within the occupied environmental spatial area (e.g. Guisan, Edwards Jr. et al. 2002, Block, Jonsen et al. 2011, Zydelis, Lewison et al. 2011). Therefore, ecological relationships between species and their resources (physical and biotic) can be determined, providing a central approach in many current analytical methods, including resource selection probability functions (RSPFs) (Manly, McDonald et al. 2002). RSPFs are a machine learning technique where the utilised habitat is modelled *versus* the unutilised but available habitat (Manly, McDonald et al. 2002, Boyce 2006, Chetkiewicz and Boyce 2009, Lele 2009). This model quantitatively characterises the probability of usage by accommodating either categorical and continuous features or variables, while facilitating the spatial structure to be incorporated with, for example, remotely sensed environmental features (Boyce and McDonald 1999). When combined with geographic information systems, RSPFs can be a powerful tool for helping to understand potential resource usage of species.

The horizontal movements of the world's heaviest teleost, the ocean sunfish (*Mola mola*) have been examined in previous studies. Despite observed slow surface swimming speeds, sunfish are active swimmers capable of extensive horizontal and vertical movements independent of ocean currents (Cartamil and Lowe 2004). These increased movement rates were verified over both fine and broader scales using spatially accurate GPS tags (Sims, Queiroz et al. 2009). Satellite tracking studies in the North Atlantic and in the north-west Pacific oceans have demonstrated that this species undertakes northward movements in spring and southward in late summer/autumn, thus displaying spatial changes consistent with a seasonal, population-level migration linked to seasonal variations of sea surface temperature and/or forage availability (Sims, Queiroz et al. 2009a, Sims, Queiroz et al. 2009b, Dewar, Thys et al. 2010, Potter, Galuardi et al. 2010). In the north-east Atlantic, the north-south migratory pattern may also be linked to increased abundance of preferred zooplankton prey at higher latitudes as waters seasonally warm (Parsons, Takahashi et al. 1984).

Diel movement patterns have been described for sunfish, with this species diving below the thermocline during daylight hours, while nocturnal dives were confined to the surface mixed layer (Cartamil and Lowe 2004, Sims, Queiroz et al. 2009, Potter and Howell 2011). Vertical distribution and space-use of marine predators are expected to change in relation to characteristics of the water column, and/or due to alterations in the depth preferences of prey. Thus, the observed movements through the water column may well be an optimal foraging strategy (Sims, Southall et al. 2008), as extensive vertical movements increase the probability of encountering higher prey concentrations at depth (Gunn, Stevens et al. 1999, Sims, Queiroz et al. 2009, Humphries, Queiroz et al. 2010). Hence, previous studies on ocean sunfish provided important insights into both horizontal and vertical movement dynamics. However, to understand habitat selection of a marine species it is important also to characterise the species' unused habitat. Robust models, including the approach employed here, have not been used in any region where sunfish have been studied, to our knowledge.

The aim of the present study, therefore, was to examine the movement and distribution patterns of satellite-tracked ocean sunfish in the dynamic habitats of the north-east Atlantic. Here, sunfish behavioural patterns are less well known due to the limited duration and low sample sizes of previous studies. We estimated the probability of habitat use by ocean sunfish in the region by applying a logistic model (RSPF) to a combination of environmental variables. Briefly, sunfish movements were integrated with SST, SST gradients (fronts), and primary productivity (Sims and Southall 2002, Sims, Queiroz et al. 2009). Moreover, the hypothesis that sunfish do not simply follow major currents (negative rheotaxis) was tested, investigating for possible ontogenetic shifts in this species rheotaxis. Finally, diel vertical migrations (DVM) and changes in these patterns in relation to the thermal structure of the water column were also investigated.

3.3 Methods

3.3.1 Tagging

Between February 2007 and October 2013 a total of 22 ocean sunfish were fitted with three different electronic tag types: pop-off satellite archival transmitters, PSATs (PAT-MK10, Wildlife

Computers); Argos Platform Terminal Transmitter, PTT (SPOT – smart position only tags, Wildlife Computers); and an integrated Fastloc-GPS^{TM} tag (Wildlife Computers and Sirtrack Ltd). The majority of sunfish were tagged in a set-net targeting tuna, off Olhão, southern Portugal, where, on a daily basis, a large number of healthy individuals are captured and released. These tags were placed in the muscle near the base of the dorsal fin prior to 2013, after which the tag was attached to the caudal fin. In addition, three individuals were tagged off Ireland, by approaching slowly in a rigid inflatable boat and throwing a cast net over the fish which was then hand captured. Off Portugal SPOT5 tags were attached to three sunfish, using stainless steel bolts, washers and nuts provided by the manufacturer. Tags were placed near the tip of the dorsal fin to maximise chances of transmission when the animal was near the surface. Another eight sunfish off Portugal were fitted with Fastloc-GPS^{TM} tags mounted in a cylindrical housing, attached externally to the fish via a 1.5 m tether of 200-lb test monofilament line and alloy crimps. The remaining 11 individuals were tagged with PSATs (Mk10) tags secured externally to the base of the dorsal fin using a 0.5 monofilament line of ~0.30 m and alloy crimps. Of these 22, four tags did not report or reported very few records and/or locations so were not considered in the analysis.

3.3.2 Ethics statement

This study was performed according to national Portuguese laws for the use of vertebrates in scientific research (Directive 113/2013, Ministério da Agricultura, do Mar, do Ambiente e do Ordenamento do Território), and all procedures followed the EU legislation Directive 2010/63/EU. Our study did not involve endangered or protected species, no animals were sacrificed, and procedures for reduction, replacement and refinement were thoroughly undertaken. Moreover, tagging was performed whilst adopting preventive mitigation techniques, such as the thoroughly sterilize of the material to minimize the potential infection risk and ensuring a predictable and relatively brief wound healing, with the procedure having a trivial impact to the animal. With regards to the tag weight and effect, previous studies reported an immediate stress response, with the tagged sunfish returning to more characteristic swimming depths after periods of 1 to 5 h (Cartamil and Lowe, 2004). Moreover, shedding has been frequently reported in externally-attached transmitters to teleost species (e.g. yellowtail, Seriola quinqueriadata, Ichihara et al., 1972; lake whitefish, Coregonus clupeaformis, Bégout et al., 1998 and the two-spined blackfish Gadopsis bispinosus, Broadhurst, Ebner et al. 2009a). However, no loss of external tags was reported on Macquarie perch Macquaria australasica after 28 days in a similar study (Broadhurst, Ebner et al. 2009b). Hence, not all species present the same results to tagging, and importantly, one should not uncritically generalise results from one species. In contrast to the tag attachment into the body of the fish, harnesses have been proven valuable (Houghton et al., 2009) for short-temporal studies in sunfish, however, potential erosion of muscles in the long run would certainly alter the fish behaviour, and eventually promote microbial infection and death of tagged fish (Jepsen et al., 2015).

3.3.3 Track processing

Argos positions were obtained for two SPOT5 tracked fish and eight Argos linked Fastloc^{TM} GPS tags and were calculated via the Doppler shift of consecutive transmissions via Argos satellites (CLS Argos). This system provides positions with variable accuracy, from 150 m (LC 3) up to 10 km (LC B -Costa, Robinson et al. 2010). To improve the location estimates, biologically implausible locations were first removed using a 5 m s^{-1} point-to-point swim speed filter, together with any points that fell on land (n = 3). Light intensity recorded by the PSAT tags at the remaining locations was used by the manufacturer's software (WC-GPE, global position estimator program suite) to determine midday or midnight to estimate longitude, and day length to estimate latitude. An inbuilt state-space model, the unscented Kalman filter – UKFsst, (Lam, Nielsen et al. 2008), was then applied to the raw position estimates in order to correct anomalous positions, using SST data from NOAA Advanced Very High Resolution Radiometer (AVHRR) 1/4° daily Optimum Interpolation Sea Surface Temperature product[1]. Thus, most probable tracks were then obtained

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[1] http://www.ncdc.noaa.gov/

after removing improbable locations (*n* = 14). Given the irregular temporal scale of both Argos and light-level data, a Kalman filter was applied to the corrected tracks, implemented in the *crawl* package in R software, interpolating all tracks into daily positions. Argos positions were parameterised with the constant (K) error model parameters for longitude and latitude implemented in the crawl package (Johnson, London et al. 2008). UKFsst geolocations were parameterised with standard deviation (SD) constants (K) which produced the smallest mean deviation from concurrent Argos positions as described by Sippel et al. (2011). To avoid inaccurate interpolations, gaps exceeding 20 days were removed and respective tracks were split into sections so no extra bias due to interpolating across large gaps was added (Block et al., 2011). Tracks were then plotted using ArcGIS geographical information system (ESRI Inc., CA, USA), where home range was estimated (95% minimum convex polygon – MCP). Spatial usage from all individuals was then analysed by means of a kernel density estimator (KDE). Here, to reduce deployment area biases, mean days per grid cell was calculated as the ratio between the total number of positions within each grid cell and the number of respective tags (Walli, Teo et al. 2009). This way, position dataset density is normalised by the tracking effort per cell and hence we objectively quantified sunfish high use regions (Walli, Teo et al. 2009).

3.3.4 Distribution per season and sunfish size

Sunfish movements were first analysed in relation to season and categorised as follows: winter (December – February); spring (March – May); summer (June – August) and autumn (September – November), with latitudinal occupancy of fish being explored regarding the time of year. A single factor analysis of variance on ranks (nonparametric Kruskal-Wallis test) examined seasonal movement differences. As fish tagged in Ireland (*n* = 3) may bias the variability found in latitudes occupied, these datasets were removed for the latitudinal displacement analysis per season. As different sized fish were tracked in this study, we also investigated the possible link of detected movement patterns in relation to the mean total length TL of fish tracked, which was 0.92 m. Thus, individual daily distances and cumulative displacements were examined per size class.

Accounting for size dependent track lengths and the apparent limited movement found after tagging (Figure S1 C), both small and large sunfish daily cumulative displacements were examined for two periods: first 25 days – initial limited movement period detected; and post-25 days up to the maximum tracking period of small sunfish.

3.3.5 Environmental integration of sunfish tracks

To integrate sunfish movements within the encountered environment, the following environmental features were analysed: sea surface temperature (SST - from AVHRR V2 NOAA Optimum Interpolation 1/4 Degree Daily Sea Surface Temperature Analysis); SST anomalies with reference to previous years for temporal variability detection; SST gradients (thermal gradients measured as the difference from the surrounding SST pixels when compared to the central, occupied pixel) and chlorophyll *a* concentration (mg/m^3) for case 1 water from MODIS Moderate Resolution Imaging Spectrometer, GlobColour level-3 Product 1/4 Degree Weekly)*,* as a proxy for primary production. Large scale PSAT tracking system associated errors were accounted for in the environmental extraction by calculating an average of the surrounding 5 pixels (5×5 matrix) for light-level locations (totalling 125 km^2 and encompassing the PSAT error of ~111 km, (Sippel, Holdsworth et al. 2011, Schaefer, Fuller et al. 2015). Afterwards, latitudinal movements of sunfish were compared to temperature (SST), frontal regions (SST gradients) and productivity (Chl *a*) along the fish’s occupied region. Briefly, we calculated average SST, Chl *a* and SST steep gradients for each latitude from 30 to 52º N in 0.25º latitudinal steps and longitudinally for the area between -12 to -2º W at the same incremental steps.

To model sunfish distribution in relation to the environment we used a resource selection probability function (logistic RSPF) with maximum likelihood estimates and nonparametric bootstrap standard errors. To account for the spatial error around real individual geolocations, we randomly resampled the sunfish location 30 times within respective tag-specific longitudinal and latitudinal Gaussian errors (0.12º for Argos following Patterson, McConnell et al. 2010) (and 1.08º latitude and 0.53º longitude in PSAT according to Sippel, Holdsworth et al. 2011). Thus, averaged environmental features from the resampled locations were assigned to each of the original corrected locations and only records with complete environmental description were maintained in the analysis. For each tracked sunfish both turning angles and step lengths were calculated and frequency distributions delineated. Based on this step, a total of 100 null tracks (pseudo absences) per real trajectory were simulated. The initial position for these null tracks was randomly set within the actual tagging location error field while accounting for each system localisation uncertainty, as stated above (Patterson, McConnell et al. 2010, Sippel, Holdsworth et al. 2011); the initial turning angle was derived from a uniform distribution. Discrete steps and turning angles were then drawn from each individual real step-length and turning angles distributions, with the former limited by the actual number of individual steps for each tracked fish. In between each position, erroneous locations on land were identified and replaced by new step length and angle and the resultant simulated tracks were constrained to the 95% home range MCP of tracked fish. To reduce the spatial autocorrelation between positions, only consecutive positions separated by a 1/3º of degree were maintained and used in the model computation. This step was performed in both real and simulated tracks, and the 1/3º was chosen to encompass the resolution of the environmental features selected.

RSPF model included SST, Chl *a*, gradients of SST and anomalies of SST, all treated as explained above. Analysis of colinearity among the selected variables was performed using Spearman’s rank correlation matrix but no significant correlation was found for the set of environmental features extracted. Best model was obtained by using Akaike values (AIC) after removing each variable at a time, using “*qpcR*” package in R, and included all variables given the absence of a null Akaike weight (wAIC) associated with each reduced model. Final models were then run as follows: (i) a general modelling of the overall sunfish habitat selection was performed using all candidate variables and sunfish records; (ii) seasonal models were run by splitting the record dataset into each season. Finally, both overall and seasonal model probabilities were mapped for visual inspection. Following Burnham and Anderson (2002), variables' significance was inspected by comparing the performance of different models from which each variable was removed sequentially , using the cross-validation property of AIC under repeated sampling (wAIC). Model overall deviance was then calculated as the ratio between the difference between the full model and the null model deviances (no variables incorporated) with this null deviance (Zuur, Ieno et al. 2009). Model validation (Hosmer and Lemeshow goodness of fit – GOF) was assessed for all generated frameworks.

3.3.6 Influence of currents on sunfish movements

Information of currents magnitude and direction (*u* and *v* components) were extracted for every daily sunfish position, using AVISO Global DT-Ref Merged MSLA – Absolute magnitude (or modulus) of the geostrophic water velocity vector, in cm/s – weekly temporal resolution. Sunfish observed speed, over the ground, was then corrected by subtracting the current component (*V. obs. = V. animal + V. current)*, following Gaspar et al., (2006). Direct comparisons of sunfish bearings and currents headings were made in a per-point analysis together with the ratio between velocities/magnitudes. Using circular statistics, daily differences between sunfish and currents' bearings, as measured from the adjacent track position, were calculated. The resultant angles were then classified into five different classes: moving with the current (0º), swimming at an acute angle towards the feature (45º and 135º), transversing the currents (90º) or swimming directly against currents (180º). Only positions with complete current information were used in the calculation. Thus, relative swimming orientation with respect to the currents direction, rheotaxis*,* were identified and tested for differences among individual sunfish sizes. In this step, a Fisher's exact test was performed to test the null hypothesis of no association between swimming angles towards currents and sunfish size.

3.3.7 Diving profiles of sunfish

Vertical profiles were derived from PSAT and one Fastloc-GPS™ satellite relayed depth data, i.e. summaries of depth and temperature utilization binned at four (PSAT) or six hours (Fastloc-GPS^{TM}), depending on the tag programming set-up. This dataset was processed using the manufacturer's software and then analysed by means of a custom written function in R software for PDT time-at-depth (TAD bins 0, 5, 10, 20, 30, 50, 70, 100, 150, 200, 250,400, 600 and >600 m) and time-at-temperature (TAT bins 2, 4, 6, 8, 10, 12, 13, 14, 15, 16, 17, 19, 21, >21 ºC) analysis. Diel periods were split following the algorithm provided by the National Oceanic & Atmospheric Administration (NOAA)[2] . Data from bins encompassing either sunrise or sunset hours were excluded from the analysis, assuring no night hours were wrongly considered in the daylight period or *vice versa*. Diel depth and temperature data were then plotted as frequency histograms by summarising both the TAD and TAT matrices into a 24 hour window by averaging the depth (or temperature) attained at every four (or six) hours. A total of 9 individuals were tracked with these depth recorders, of which only two had lengths greater than the average of 0.92 m in this study, which hampers statistical analysis per sunfish size class. However, correlations (Spearman rank correlations, as data failed normality test) of time spent at deep layers (> 250m) and surface (≤5m) were run per individual size to inform on possible size differences in vertical usage. In addition, using information recorded on both minimum and maximum depth (from the retrieved PDT data) the vertical extent was summarised per individual sunfish. Spearman correlations were also computed to determine if water column occupancy amplitudes varied with sunfish sizes.

To detect changes in depth occupancy, the TAD matrix was investigated using a modified version of the split moving window (SMW) method to detect significant shifts in time-at-depth data to identify behavioural phases (Queiroz, Humphries et al. 2010). This method comprises a variable-sized split window with temporal scales ranging from four hours up to five days to calculate dissimilarities between the two halves of the window along the time-steps of the vertical track.

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[2] http://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html.

Euclidean dissimilarity between different halves is then assigned to the centre of the window. The process is repeated moving one step forward, until the window reached the end of the data series. Statistical significance of dissimilarities for each central point of each window is then calculated; the boundary location wherever a significant shift was detected denotes where the depth profile should be split (see Humphries, Queiroz et al. 2010, Queiroz, Humphries et al. 2012). Diel vertical habitat use (TAD and TAT) was then analysed per sunfish trajectory section. To compare daytime and night-time temperature preferences within each SMW section, we used non-parametric two-sample Kolmogorov–Smirnov (K–S) tests for the TAT obtained, in R. Lastly, retrieved PDT data of simultaneous depth and temperature information at each temporal interval (4 or 6 hours) was used to characterise sunfish occupied water column (revealed by the variance of temperature with depth). Briefly, averages derived from the minimum and maximum temperatures recorded for each depth bin occupied within the 4 or 6 hours summarised dataset, were calculated. Final means and standard deviations for each depth recorded (m) with associated temperature were then computed, pooled from each of the sunfish SMW determined section.

3.4 Results

From 2007 to 2013, 22 ocean sunfish were satellite tagged of which 18 (ranging in sizes from 0.60 to 1.40 m total length (TL); mean 0.92 m, Table 5) successfully reported sufficient data for movement analysis. Of these, eight individuals were tracked with pop-off archival satellite transmitter (PSAT) tags able to store depth, temperature and light-level data, providing both horizontal and vertical profiles. PSAT tags provided 9 – 43 geolocations per individual (mean 21) and tracking times varied between six and 120 days (mean 55 days). To estimate the spatial inaccuracy of the UKFsst corrected tracks, both longitude (σ_x) and latitude (σ_y) standard deviations obtained from the parameterised geolocation errors were calculated for the pooled data (Lam, Nielsen et al. 2010). The median error associated with the estimated longitude and latitude corrections were 0.49º [0.31 to 1.19º] and 1.19º [0.94º to 1.96º], respectively. Thus, sunfish PSAT tracks had similar spatial errors to those found previously (e.g. Sippel, Holdsworth et al. 2011, Schaefer, Fuller et al. 2015).

The remaining 10 fish were tracked via Argos receivers on polar-orbiting satellites, with one tag (#14) also recording depth and temperature data (see Methods). Of the obtained Argos locations, 60% had location classes (LC) 1-3 with reported error fields < 1.5 km. Argos tagged fish were at liberty for 5 – 172 days (mean of 66 days), with the total number of geolocations per individual ranging from 18 up to 226 (mean 94). On average, the number of daily gaps (days) was 2.43, ranging from 1 up to 69 days, with only two tracks having gaps longer than 20 days (Table 7). From 0.2 up to 10 positions per day were obtained for tracked sunfish (with an average of 1.6; see Table 7 for details).

Tracking location numbers during the year were not consistent, with 28% of the retrieved positions corresponding to autumn; 29% to spring; 32% to summer and 11% to winter months, the season with the fewest sunfish positions. Moreover, on average, Argos tags remained attached for longer (96 days versus 58 and 55 from Argos-GPS and PSAT, respectively) and provided the largest average number of positions (103 against 91 Argos-GPS and 20 of PSAT). However, if weighted by days at liberty, Argos-GPS outperformed, in number of locations per tracked day (~3), both PSAT (0.5) and Argos (1) tags.

Table 5 Summary of all 22 sunfish tagged individuals in this study.

Type	Id	PTT	Tagging date	Pop-up date	Tagging location	Total length (m)	Days liberty	Final date	Geolocations (N)
PSAT	1	66943	28/02/2007	29/04/2007	Portugal	0.70	43	12/04/2007	17
PSAT	2	66944	28/02/2007	29/05/2007	Portugal	0.70	91	30/05/2007	18
PSAT	3	40401	08/08/2007	06/11/2007	Ireland	0.64	54	01/10/2007	35
PSAT	4	40398	12/08/2007	11/10/2007	Ireland	0.66	8	20/08/2007	11
GPS	5	75760	14/05/2008	-	Portugal	0.60	15	29/05/2008	23
GPS	6	75761	14/05/2008	-	Portugal	0.60	5	19/05/2008	50
PSAT	7	86398	21/08/2008	18/01/2009	Ireland	0.63	47	07/10/2008	15
GPS	8	75763	06/11/2008	-	Portugal	1.00	95	09/02/2009	142
GPS	9	75762	18/05/2010	-	Portugal	0.97	172	06/11/2010	125
GPS	10	99102	21/05/2010	-	Portugal	1.00	9	30/05/2010	18
PSAT	11	107083	03/04/2012	01/08/2012	Portugal	0.80	41	14/05/2012	12
PSAT	12	107087	03/04/2012	01/08/2012	Portugal	0.75	34	07/05/2012	9
SPOT	13	66959	30/04/2012		Portugal	0.93	64	03/07/2012	57
GPS	14	15122	18/05/2012	18/05/2013	Portugal	100	51	08/07/2012	59

SPOT	15	66960	09/05/2013		Portugal	1.40	127	13/09//2013	148
PSAT	16	107085	10/05/2013	07/09/2013	Portugal	1.00	120	07/09/2013	43
GPS	17	133671	14/10/2013	-	Portugal	1.25	73	26/12/2013	89
GPS	18	133672	19/10/2013	-	Portugal	1.15	47	05/12/2013	225
PSAT		86397	14/05/2012	11/10/2012	Portugal	0.93	-	DNR	
PSAT		86408	03/05/2013	31/08/2013	Portugal	0.85	-	DNR	
SPOT		40391	08/05/2013	-	Portugal	1.05	-	PT	
PSAT		85693	09/05/2013	06/09/2013	Portugal	1.35	-	DNR	

PT- Poor transmission; DNR- Did not report.

Table 6 Summary of tag type performance

		Geolocations	**Days at liberty**	**Pos. per days at liberty**
GPS	AVERAGE	91.38	58.38	2.86
	MAX	225.00	172.00	10.00
	MIN	18.00	5.00	0.73
PSAT	AVERAGE	20.00	54.75	0.48
	MAX	43.00	120.00	1.38
	MIN	9.00	8.00	0.20
SPOT	AVERAGE	102.50	95.50	1.03
	MAX	148.00	127.00	1.17
	MIN	57.00	64.00	0.89
All	AVERAGE	60.89	60.89	1.60
	MAX	225.00	172.00	10.00
	MIN	9.00	5.00	0.20

Table 7 Summary of all 18 sunfish tracked in terms of daily gaps

			GAPS			
PTT	**≥ 3d**	**≥ 5d**	**≥ 10d**	**≥ 20d**	**Min. Gap**	**Max. Gap**
66943	5	4	2	0	0	11
66944	8	5	2	1	0	42**
40401	5	2	0	0	0	8
40398	0	0	0	0	0	1
75760	2	1	0	0	0	5
75761	1	1	1	0	0	12
86398	6	5	1	0	0	10
75763	11	1	0	0	0	9
75762	20	14	2	0	0	17
99102	1	0	0	0	0	9
107083	6	3	1	0	0	10
107087	4	2	1	0	0	13
66959	8	4	1	0	0	10
15122	1	0	0	0	0	3
66960	5	1	1	1	0	69*
107085	14	8	2	0	0	11
133671	10	2	1	0	0	10
133672	3	0	0	0	0	4

** Gaps exceeding 20 days were excluded by splitting tracks when gap in middle of track (*) and removed completely when at beginning/end (**).*

3.4.1 Distribution, horizontal movements and seasonality

Overall, tracked sunfish displaced between 17 and 754 km from tagging locations (mean 317 ± 225), covering estimated total distances between 109 and 3351 km (mean 777 ± 874 sd). Visual inspection of the trajectories revealed a wide dispersal pattern in the region (Figure 7A), which was related to season, with a northern latitudinal movement evident in spring-summer and southern movement into warmer waters of the Mediterranean or the north-west African coast observed during the colder months of winter (Figure 7B). Furthermore, tagged fish in northern latitudes off Ireland all moved south in late summer and autumn months (Figure 7B).

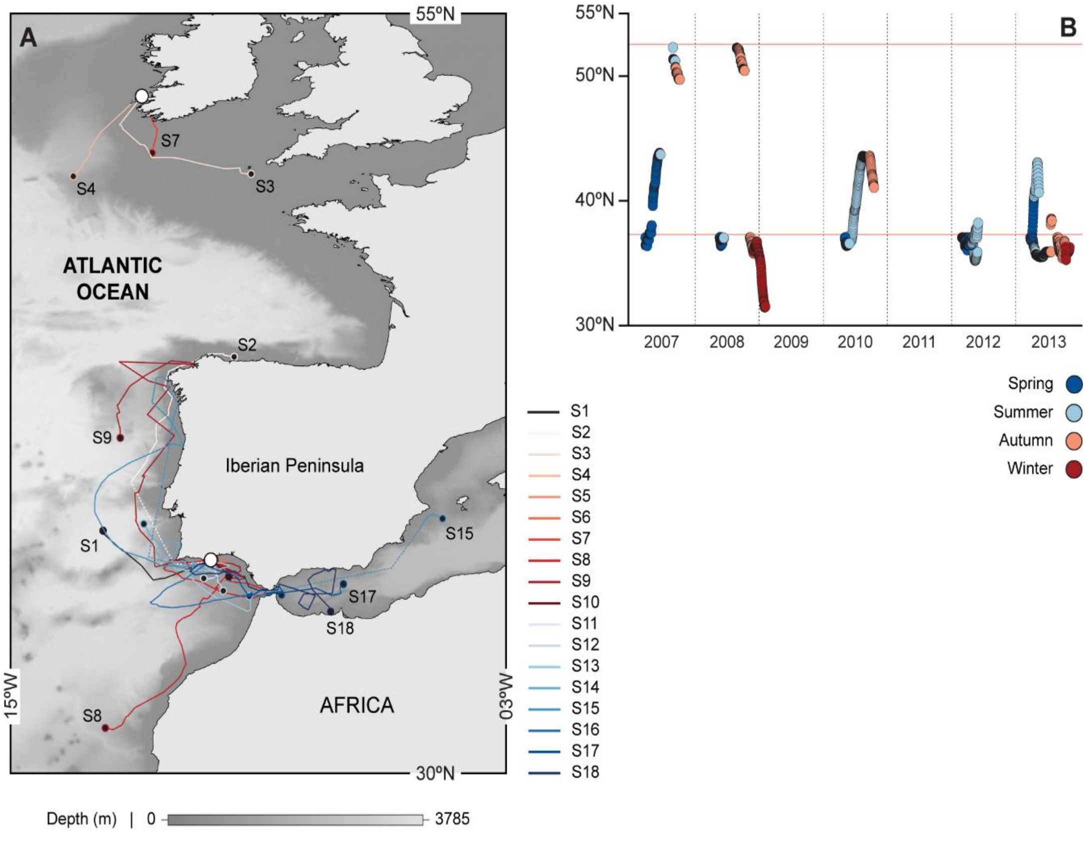

Figure 7 Mapping of tracked sunfish in this study (A) and latitudinal components of positions per day (B). Sunfish tracks individually coloured S1 – S18 overlaid on bathymetric map in A. White dots denote tagging locations. Seasonal latitudinal movements depicted in B dark blue-spring; light blue- summer; pink- autumn and red- winter.

Moreover, even after removing sunfish tagged off Ireland, that likely bias the seasonal latitudinal differences found, significant differences in distribution by sunfish per season (Kruskal-Wallis *H* = 158.1, df = 3, *p*<0.001) were found. Noticeably, the widest latitudinal range was observed both in summer and autumn (increased standard deviations: autumn mean 38.56º N ± 3.205º; summer 38.72º N ± 2.498º; spring 37.64º N ± 2.094º; winter 35.02º N ± 1.64º), reflecting an expanded north-south distribution. On the other hand, when accounting for the tagging bias (by dividing the number of locations retrieved per number of tags deployed in each 25km grid cell) we found that besides this wide distribution and the seasonality in movements, tracked sunfish also displayed high space-use focused in three particular areas (Figure 8A). Thus, despite reducing the tagging bias, sunfish densities (estimated by the KDE on the normalised tracked positions) were still found predominantly in the Gulf of Cadiz region, north-west Iberian and in the Alboran gyre.

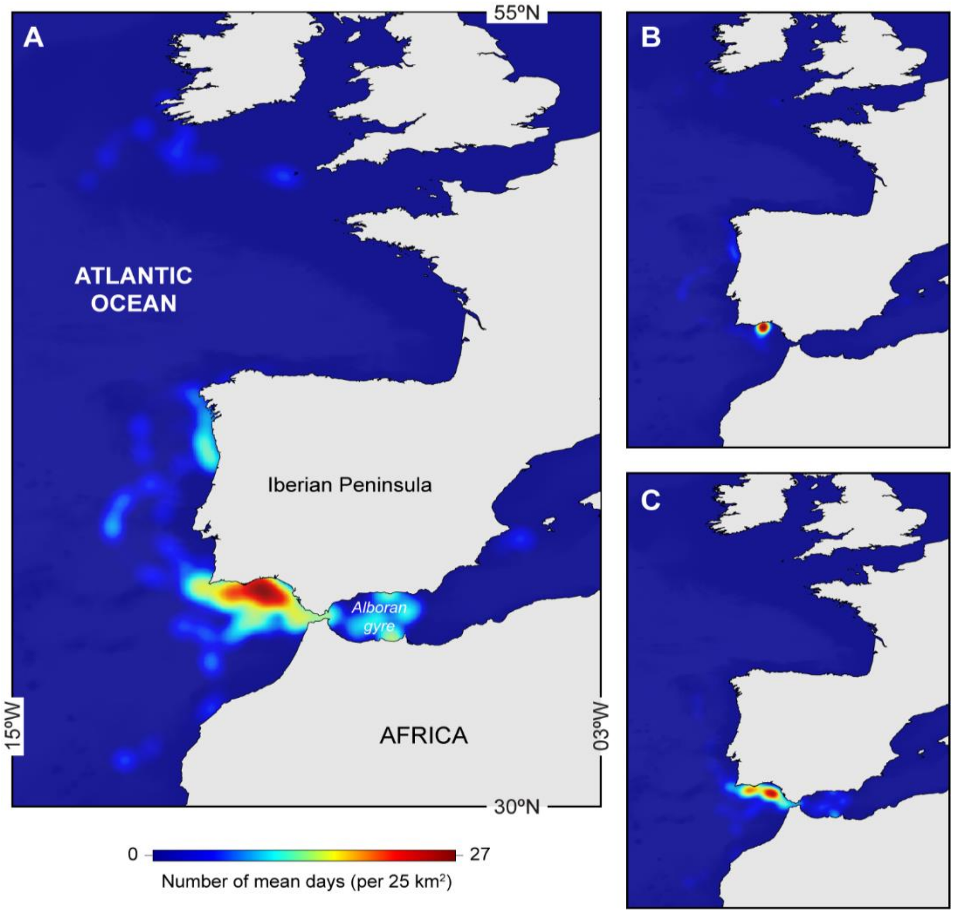

Figure 8 A Kernel density estimation (KDE) of sunfish occurrence; B KDE of individuals of total length (TL) below the average 0.92m; C larger sunfish KDE.

Interestingly, the majority of sunfish tagged in spring off southern Portugal that did not display northward movements (*n* = 6 out of 8) were individuals smaller than the average TL of all tracked sunfish (0.92 m). Hence, a size-based analysis was completed for both the distance from tagging (Figure S1A) and daily displacement (Figure S1B). Individual analysis revealed that sunfish larger than the average TL of 0.92 m displayed significantly higher daily displacements (12570 ± 5920 m) than smaller individuals (8690 ± 4320 m) (*t* –test: *t* = 5.55, df = 157, *p* < 0.001*).* This generally greater daily distance travelled by larger individuals was also confirmed by the difference in the (cumulative) distance moved after tagging for the first 25 days, and when different tracking lengths with size and the initial limited movement period detected (Figure S1C) were both accounted for. Specifically, smaller individuals dispersed less overall (93200 ± 38500 m) than larger sunfish (128600 ± 57200 m) (*t* test: $t = -2.61$, df = 23, $p < 0.05$, Figure S1 C). This difference was maintained when analysing distances for the period post-tagging after those 25 days and up to the maximum days-at-liberty of the small fish (65 d) (*t* test: $t = -3.05$, df = 59, $p < 0.05$). Hence, even though smaller fish were tracked for shorter periods of time, these results suggest larger sunfish displaced farther from the tagging location.

3.4.2 Environmental integration of sunfish movements

Plots of sunfish latitudinal movements along the mean coastal SST showed that tagged fish experienced approximately the same thermal range, independent of latitude, time within the year, and of year, with the exception of 2007. Overall, sunfish tracked outside the Mediterranean experienced a SST range from 13º to 23º C. This thermal envelope was found to be warmer for individuals that entered the Mediterranean (16º to 27º C, Figure 9 and Figure 10). Sunfish also generally occupied waters of low productivity (< 1 mg/m^3 chlorophyll *a* concentration, where chlorophyll *a* is a proxy for primary production), with sporadic encounters with productive 'hotspots' (from 5 to 16 mg/m^3). The integration of sunfish movements with chlorophyll *a*, also showed that although not tracking higher productivity regions, sunfish seem to avoid oligotrophic 'coldspots' (Figure 9). Lastly, especially during summer months and except for the years 2007-2008, sunfish movements appear linked to maxima in temperature gradients. The preference for these thermal discontinuities (fronts) or the vicinity of these, was clearer in the sunfish movements made towards the Mediterranean (Figure 9). Thus, overall movements appear to generally 'track' a strict thermal envelope of SST and follow seasonally persistent thermal gradients, whilst avoiding oligotrophic regions.

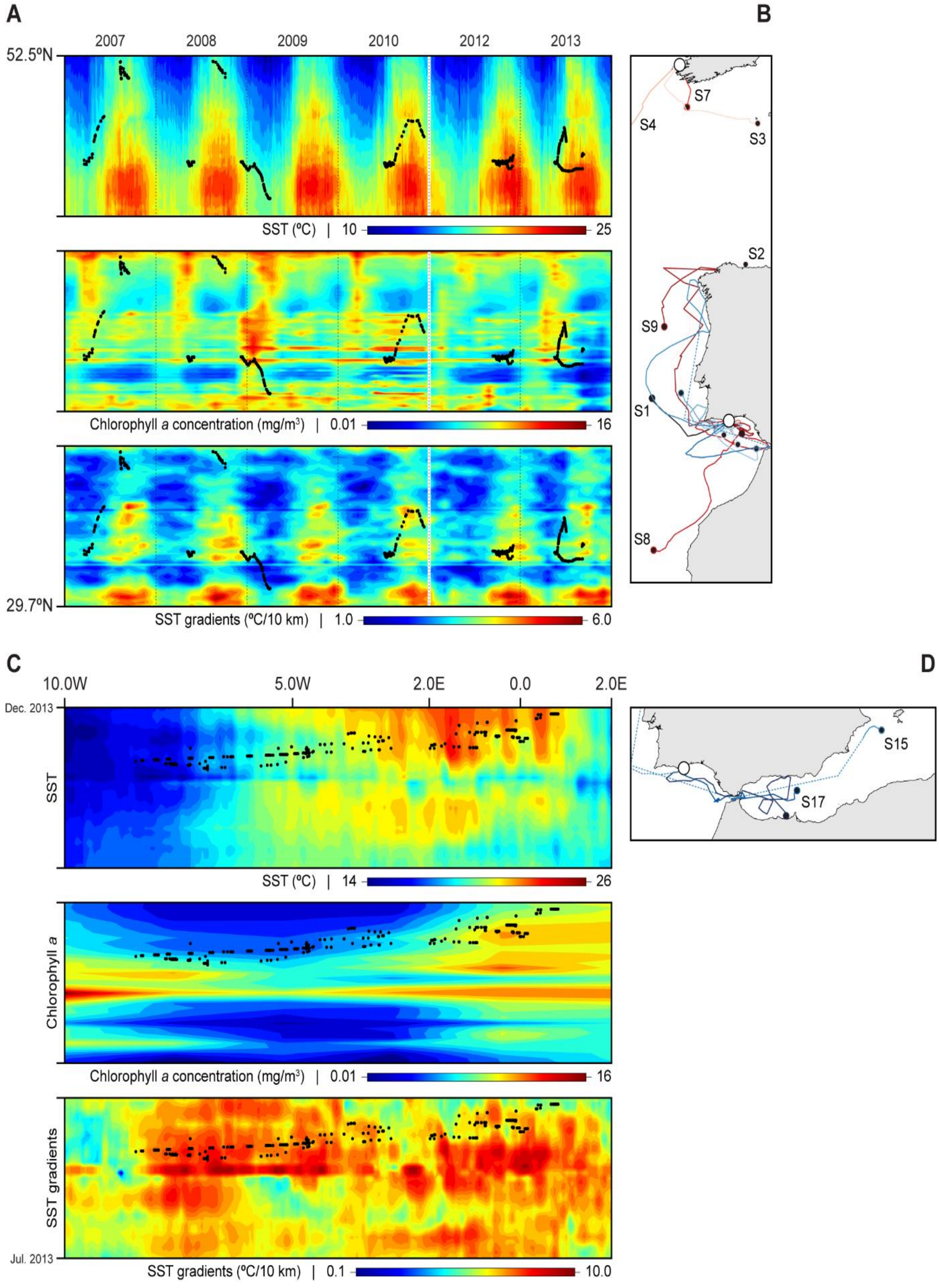

Figure 9 Coastal environmental integration of tagged sunfish A) latitudinal and C) longitudinal movements. Retrieved positional components were overlaid on monthly averages of sea surface temperature, productivity and thermal gradients at 0.25º increments from the coast. Right panels

with maps of respective tracks for comparison; B) latitudinal tracks along the Iberian Peninsula and D) longitudinal tracks into the Mediterranean.

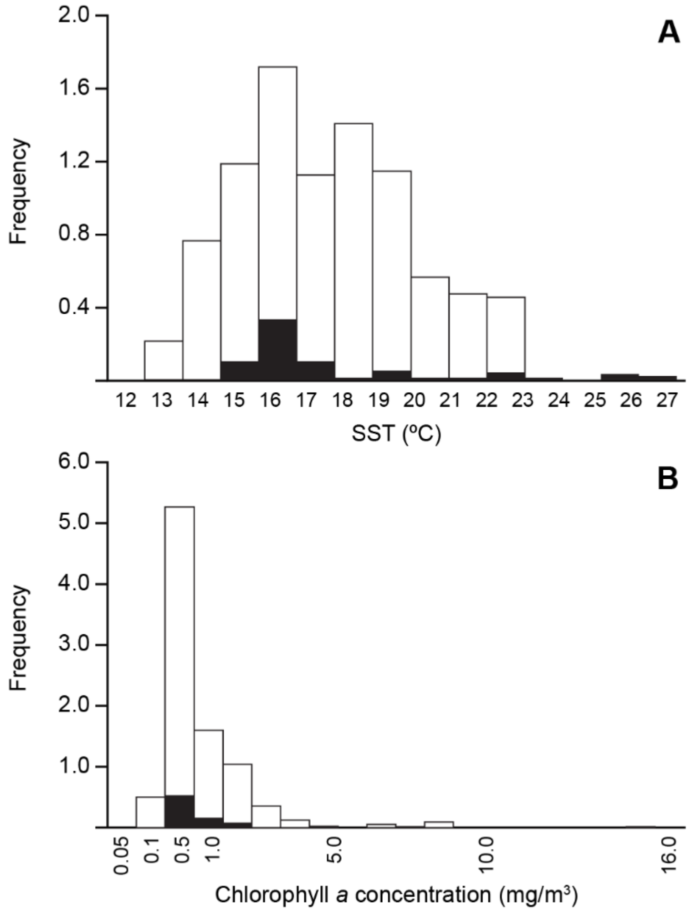

Figure 10 Encountered SST and chlorophyll *a* histograms for all sunfish tracked locations. Black denote tracks when in the Mediterranean (#14, #17-18) and white all the remainders.

3.4.3 Sunfish habitat preferences

To investigate the influence of the environment on sunfish movements, tracked fish selected habitat selected was compared to that available in non-occupied regions, thus providing predictive maps of sunfish occurrence in areas where they were not tracked. Overall, the most parsimonious RSPF model included all four variables (SST, SST gradients, SST anomalies and chlorophyll *a* concentration) (Table 8 and Figure 11). Model was validated by the GOF (Hosmer and Lemeshow) χ^2=12.22, df = 8, *p* = 0.142. This model explained 33 % of the sunfish habitat-use probabilities and variables were selected based on Akaike weights (Burnham and Anderson 2002) which specified the importance of each parameter for the model. Briefly, using the cross-validation property of AIC under repeated sampling, the full model has greater chances of being selected (45% of the time), whereas a model without chlorophyll *a* was ranked best 27% of the time and a model without SST anomalies would better inform sunfish habitat selection for 28% of the times. Importantly, models with no SST or SST gradients had negligible probabilities of explaining the fish distribution (Table 9). Therefore, sunfish habitat use was found to be strongly influenced by temperature, with fish avoiding higher temperatures, preferring areas with sharp SST gradients and positive anomaly with regards to previous years, whereas chlorophyll *a* had no significance for the fish overall habitat selection.

Summer and autumn seasonal models were significantly influenced by frontal regions approximated by SST gradients, which was confirmed by the low wAIC when this feature is removed from the full models. With regards to chlorophyll *a*, although not significant, the negative effect was maintained in summer, spring and autumn months, while SST negatively influenced sunfish habitat during summer and spring months. Despite being a significant driver of sunfish distribution in the general model, SST anomalies did not significantly influence the fish habitat selection seasonally. In winter, no variable was found to influence sunfish habitat directly (Table 10). Ultimately, the seasonal predictive maps of sunfish habitat not only highlighted the north-south migratory pattern across much broader scales than were visited by satellite-tracked fish (Figure 11B – E), but also identified areas likely to support high sunfish densities, even though none were tracked in those areas. For example, Figure 11C indicates a high probability of sunfish habitat selection off the southwest peninsula of the United Kingdom which in previous studies has been shown to have increased sunfish abundance in summer months (Sims and Southall 2002, Leeney, Witt et al. 2011). This indicates that satellite track-informed environmental modelling can yield species seasonal distribution maps that show heterogeneities beyond the data used to parameterise the models (see Table 11 for GOF results).

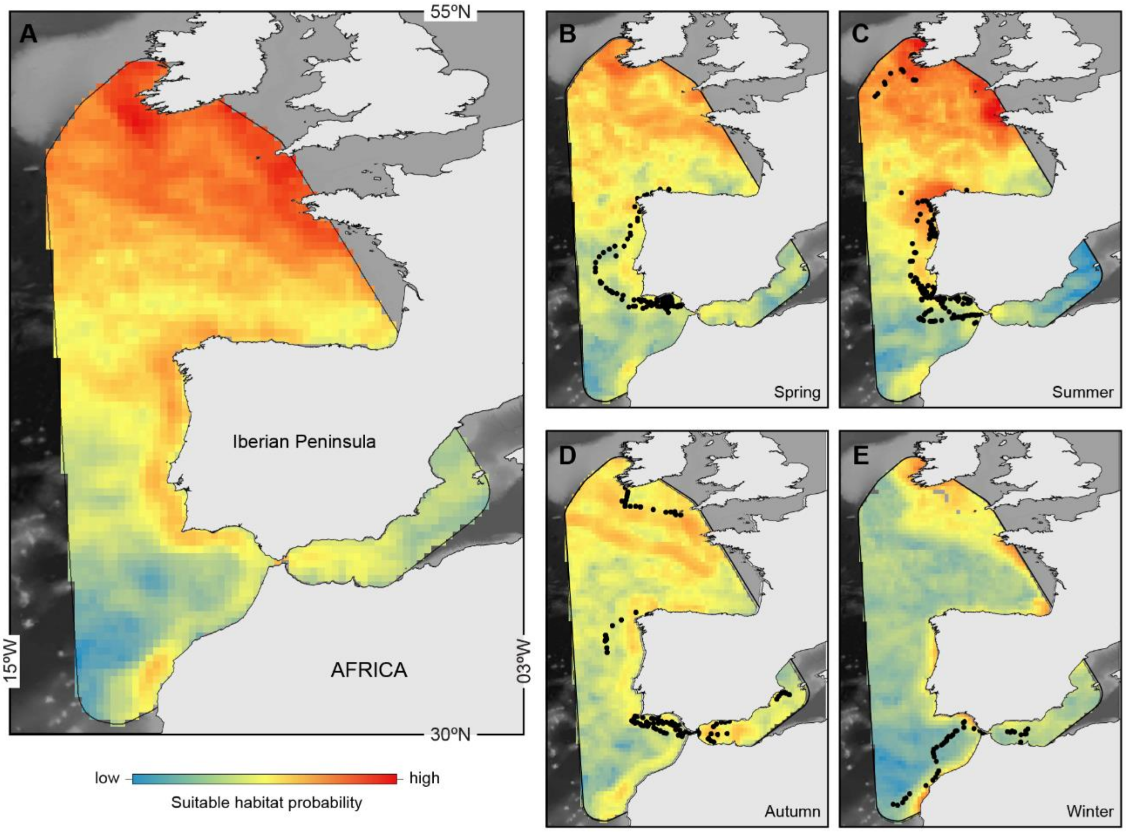

Figure 11 A) Final model predictions for sunfish distribution in the north-east Atlantic, for the tagging years and as a direct response to averaged sea surface temperatures, fronts and chlorophyll *a*; Seasonal predictive models for sunfish distribution, B) spring; C) summer; D) autumn; and E) winter.

Table 8 Coefficients of the overall RSPF model of sunfish distribution. Model coefficients are shown for each of the four variables selected for modelling the probability of occurrence of sunfish, within the species 95% MCP based on tracked individuals.

	β	**SE**	**z value**	***p value***	**exp(β)**	**2.50%**	**97.50%**
(Intercept)	17.414	3.837	4.538	5.67E-06	3.65E+07	9.893	24.934
SST	-0.526	0.111	-4.728	2.26E-06	0.590	-0.744	-0.308
Chlorophyll *a*	-0.264	0.182	-1.445	0.1484	0.770	-0.621	0.094
Slope SST	4.104	0.951	4.315	1.60E-05	60.580	2.240	5.968
SST anomalies	0.609	0.286	2.130	0.0332	1.840	0.049	1.169

Table 9 Relative importance of each variable to the final model. Step-wise models were generated excluding each variable at a time and deviances were calculated informing on the significance of each parameter to the full model (deviance, AIC, Δdeviance, ΔAIC, p value, relative likelihood and wAIC). Clearly, gradients of SST and SST were the most influent variables providing the highest % of significant variability explained (Δdeviance) and both features had the lowest wAIC if removed.

	Deviance	**AIC**	**Δdeviance**	***p value***	**ΔAIC**	**relat. LL**	**wAIC**
Full	3197.4				0	1.000	0.452
No SST	3230.2	3238.2	32.8	6.22E-11	40.750	1.42E-09	6.41E-10
No Chl a	3190.5	3198.5	6.9	0.080	1.061	0.588	0.266
No SST slope	3227.2	3235.2	29.8	2.94E-10	37.715	6.46E-09	2.92E-09
No SST Anomalies	3190.4	3198.4	7.0	0.086	0.946	0.623	0.282

Table 10 Seasonal model coefficients output. Individual variable importance also shown depicted by the wAIC column of each model.

	Parameter	**β**	**SE**	**z value**	***p value***	**Model**	**Deviance**	**AIC**	**Δdeviance**	***p value***	**ΔAIC**	**relat. LL**	**wAIC**
Summer	**Intercept**	-2.473	0.385	-6.430	1.27E-10	**Full**	1011.8	1021.8			1.613	0.45	0.22
	SST	-0.288	0.041	-7.001	2.55E-12	**No SST**	1051.4	1059.4	39.60	3.12E-10	39.212	0.00	0.00
	Chlorophyll *a*	-0.062	0.094	-0.663	0.5070	**No Chl *a***	1012.2	1020.2	0.39	0.534	0.000	1.00	0.49
	SST Gradients	1.461	0.403	3.625	0.0003	**No SST slope**	1024.0	1032.0	12.18	0.000	11.794	0.00	0.00
	SST Anomalies	0.206	0.169	1.216	0.2240	**No SST anomalies**	1013.2	1021.2	1.43	0.231	1.047	0.59	0.29
Spring	**Intercept**	27.533	10.509	2.620	8.79E-03	**Full**	915.8	925.8			1.090	0.58	0.16
	SST	-0.782	0.461	-1.697	8.98E-02	**No SST**	920.4	928.4	4.59	0.032	3.678	0.16	0.04
	Chlorophyll *a*	0.139	1.723	0.080	0.9359	**No Chl *a***	916.7	924.7	0.91	0.339	0.003	1.00	0.27
	SST Gradients	7.148	3.783	1.890	0.0588	**No SST slope**	916.8	924.8	1.06	0.304	0.146	0.93	0.25
	SST Anomalies	2.082	2.626	0.793	0.4280	**No SST anomalies**	916.7	924.7	0.91	0.340	0.000	1.00	0.27
Autumn	**Intercept**	19.148	6.765	2.831	4.65E-03	**Full**	861.3	871.3			0.371	0.83	0.33
	SST	-0.472	0.175	-2.700	0.0693	**No SST**	873.8	881.8	12.48	0.000	10.848	0.00	0.00
	Chlorophyll *a*	-0.603	0.349	-1.729	0.0839	**No Chl *a***	863.7	871.7	2.36	0.125	0.728	0.69	0.27
	SST Gradients	5.899	2.207	2.673	0.0075	**No SST slope**	896.1	904.1	34.77	3.71E-09	33.140	0.00	0.00
	SST Anomalies	1.042	0.587	1.774	0.0760	**No SST anomalies**	863.0	871.0	1.63	0.202	0.000	1.00	0.40
Winter	**Intercept**	-0.681	3.287	-0.207	0.8360	**Full**	369.0	379.0			1.995	0.37	0.10
	SST	-0.145	0.162	-0.897	0.3700	**No SST**	369.8	377.8	0.80	0.370	0.797	0.67	0.18

	Chlorophyll *a*	0.024	0.363	0.067	0.9460	**No Chl *a***	369.0	377.0	0.00	0.945	0.000	1.00	0.27
	SST Gradients	0.564	0.641	0.881	0.3780	**No SST slope**	369.8	377.8	0.77	3.80E-01	0.766	0.68	0.18
	SST Anomalies	-0.123	0.485	-0.254	0.7990	**No SST anomalies**	369.1	377.1	0.06	0.800	0.060	0.97	0.26

Table 11 Statistical output from the seasonal models goodness of fit, validation of each model.

	Winter	**Summer**	**Spring**	**Autumn**
GOF	χ2 = 0, df = 8, *p* > 0.05	χ2 =15.22, df = 8, *p* > 0.05	χ2 =20.42, df = 8, *p* > 0.05	χ2 = 5.538, df = 8, *p* > 0.05

3.4.4 Influence of currents on sunfish movements

In the overall analysis, sunfish displayed directed movements for the majority of the time, independent of the direction of oceanic currents (examples in Figure 12). Statistically we found that sunfish directions were significantly different from the headings of the currents fish were experiencing (Watson's two-sample test of homogeneity $U^2 = 0.7288$, critical value = 0.187 and $p < 0.05$). Information on currents for every daily position revealed a highly variable encountered environment, with sunfish experiencing both favourable (same direction) and opposite geostrophic currents (Figure 12 for illustrative tracks with the directionality and magnitude of currents depicted along the trajectories made by the sunfish). Remarkably, all the longer movements off the western Iberia were undertaken in summer months, a season marked by strong southwards currents, as it is visible in Figure 13.

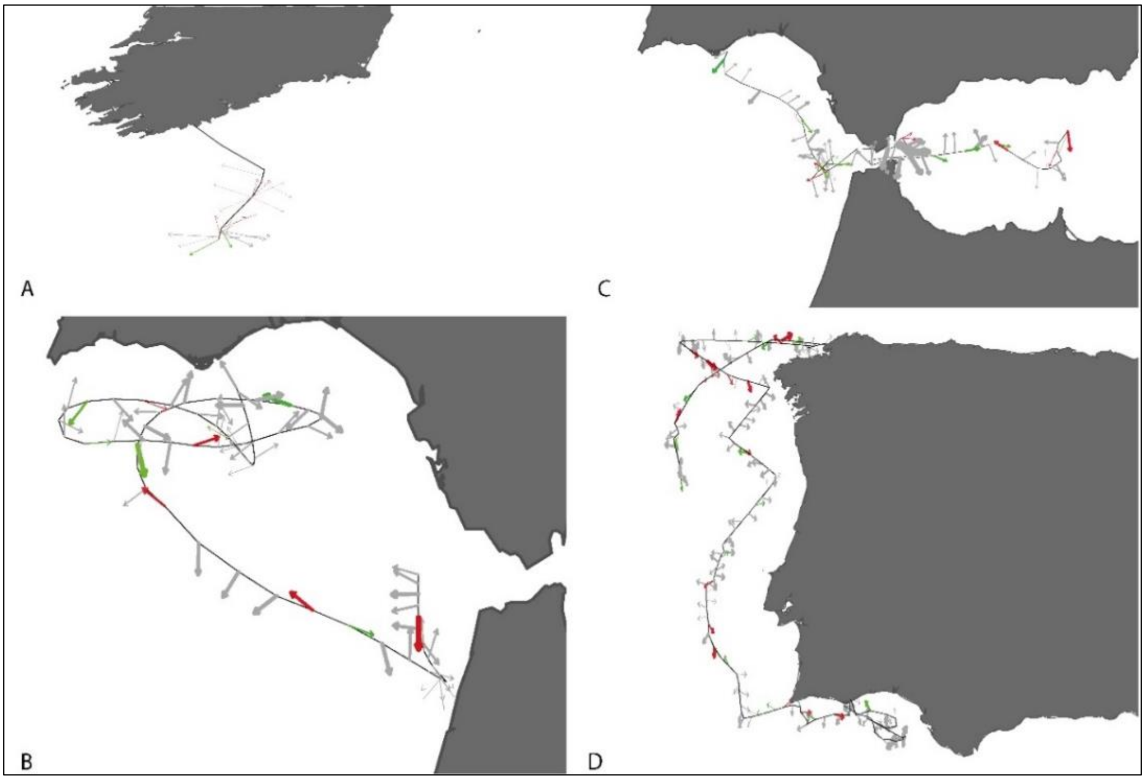

Figure 12 Examples of sunfish tracks (small sunfish A-B; larger individuals C-D), with current direction vectors overlaid (green – similar direction; red – opposite direction) and respective magnitude represented by arrow stroke width.

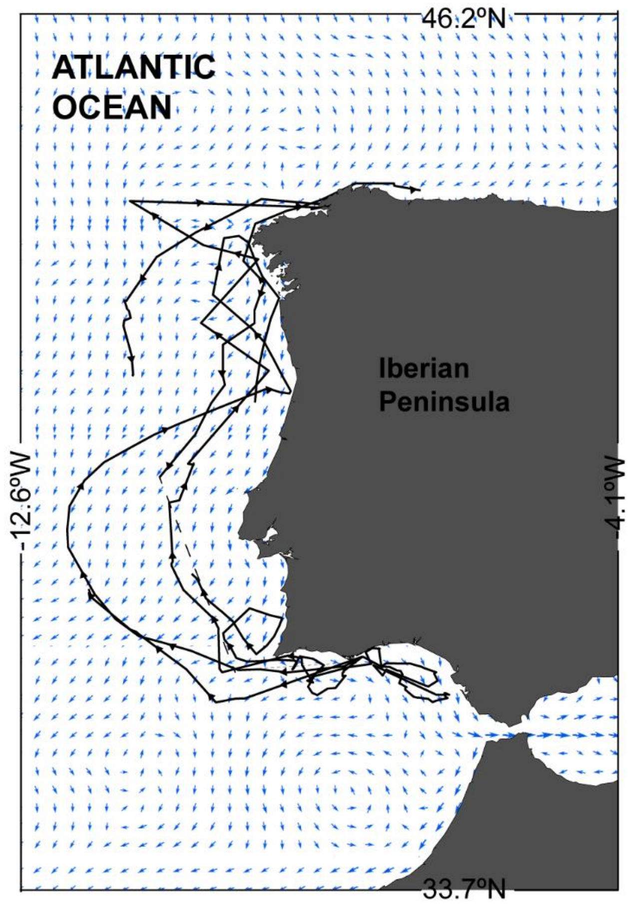

Figure 13 Map of the five longer sunfish tracks along the western Iberia, overlaid on the average summer currents direction for the tracking period, 2007-2012. Hourly data was extracted and summer (June-August) composites were calculated (using both *u* and *v* components). Arrow width represents the magnitude of the currents in place. Sunfish movements are independent of surface currents' directionality, with individuals swimming in the opposite direction to currents during summer months.

Ocean currents were detected to be highly variable, reflecting the dynamic habitats sunfish inhabited (Figure 14A). As the majority of sunfish that displaced largely from the tagging area were individuals of TL above the average size, we compared the individual bearings among size classes and found a difference (Watson $U^2 = 0.0253$; critical value = 0.187 $p < 0.05$). Moreover, although no preferred direction was observed in sunfish headings according to size, a larger difference between average bearings in larger individuals was detected (Figure 14B). This suggests a more pronounced positive rheotaxis in larger sunfish when compared to smaller individuals.

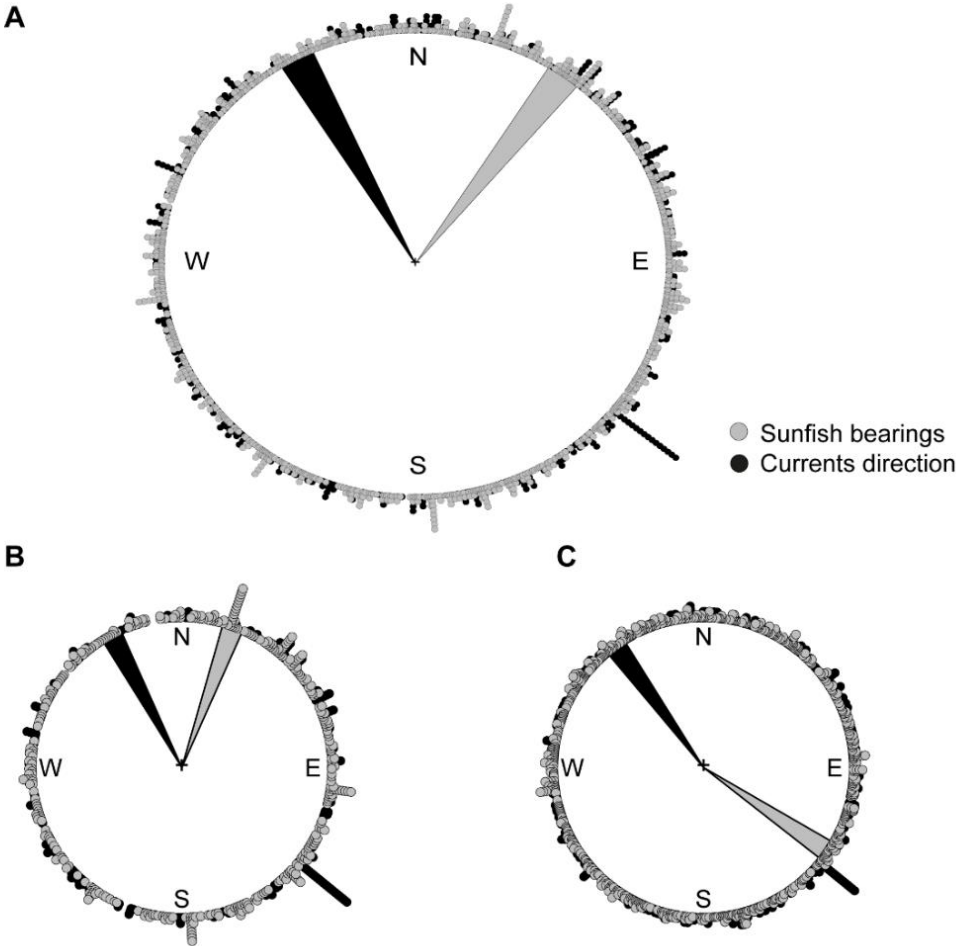

Figure 14 Rose diagrams showing A) sunfish averaged direction in comparison to current headings; B) difference attained by smaller (< 0.92m) and C) larger individuals (> 0.92m).

Table 12 Resultant angle classes between sunfish directionality and currents' headings, per size class.

	0°	45°	90°	135°	180°
Big	0.193	0.333	0.185	0.194	0.096
Small	0.218	0.342	0.245	0.113	0.082

Thus, to test this variation along the track as a function of currents' direction, bearings of fish were directly compared with the direction of currents (see Figure 15 for a plot of the difference among sunfish sizes (larger – smaller) frequency per angle class). This comparison revealed a clear size distinction with smaller individuals swimming less often against currents (angles 180°), whereas larger sunfish frequently travelled opposite to the direction of this feature.

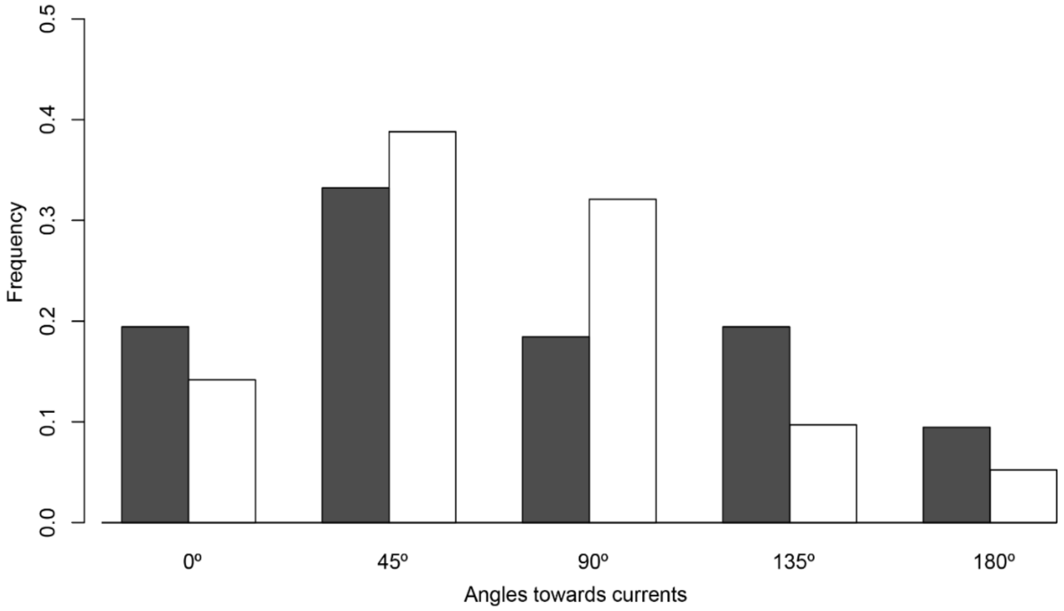

Figure 15 Barplot of the difference in resultant angles, (0º; 45º, 90º; 135º, 180º), between sunfish bearings and currents direction, per sunfish sizes (black: larger individuals; white: smaller fish).

The size specific differences in resultant angle frequencies (Figure 15) conform to the higher propensity of larger individuals to swim more frequently against major currents. Interestingly, the same amount of time (54% and 56%, for small and larger sunfish respectively) was spent swimming with the current direction (≤45º); whereas a higher proportion of larger individuals (29%) was found swimming against these features (≥135º), against only 19% of the smaller individuals [Fisher's exact test for count data with simulated *p-value* (based on 1e+07 replicates) $p < 0.05$].

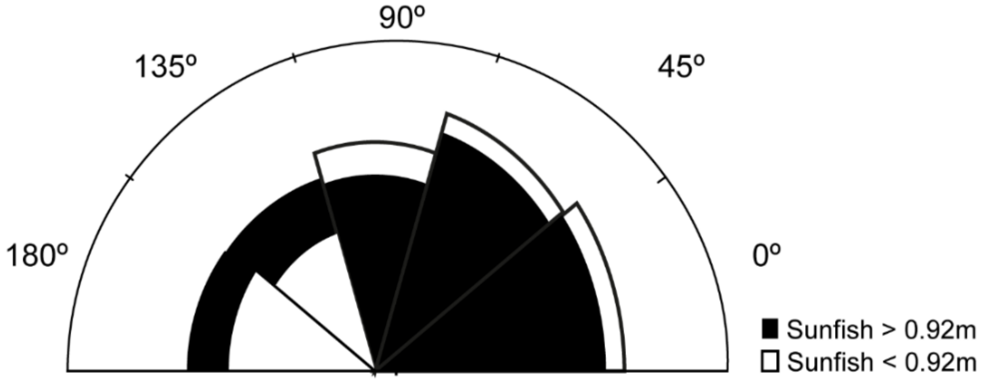

Figure 16 Proportion of resultant angle of sunfish bearings in relation to currents, per fish size class. Note the increased frequency of larger angles (135 – 180º) in larger individuals.

3.4.5 Diving profiles of sunfish

PSAT tracked sunfish exhibited an extended vertical distribution, from the surface to a maximum depth of 704 m (480 ± 125 m). Analysis of the TAD revealed that overall, sunfish spent ~25% of the time in the top 10 m layer; ~53% between 10 and 100 m depth, and 12% in depths exceeding 200 m (Figure S2). In relation to individual size, we found that larger sunfish tended to spend significantly more time in deeper layers (below 250m) (Spearman rank correlation: r_s =0.71, p = 0.03). Furthermore, we also found a positive correlation between the vertical extent of the water column used (maximum – minimum depth) and the total length of tagged individuals (Spearman rank correlation: r_s=0.710, $p < 0.05$).

3.4.5.1 Behavioural shifts in depth occupancy

The split moving window (SMW) analysis detected significant discontinuities in depth use along the individual diving profiles, which were then divided at these discontinuities into 19 sections that likely represent bouts of differing behaviour. All sections' TADs were then examined to identify periods of either normal diel vertical migration (nDVM, where the animal is in deeper water during the day, and shallower at night) or reverse DVM (rDVM, deep during the night, shallower during the day). Of the 19 sections, 10 exhibited diel changing movements, with nDVM 42 % of the time and rDVM 11 % of the time (Figure 17A-B). To our knowledge, this is the first time rDVM has been described for sunfish. The remaining 9 sections showed no difference in diel movements. Overall, 32% of the time sunfish showed a surface oriented pattern, without deep incursions (Figure 17C), whereas for the remaining 15% of the time it was not possible to assign a specific behaviour as fish exhibited similar depth occupancy both day and night, but were not restricted to surface layers (Table 13). Furthermore, no correlation between sunfish diel behaviour (normal, reverse or surface oriented) and a specific geographic region was detected and examples of the three diel diving patterns are given in Figure 6. Variability of temperature with depth, retrieved from the PDT data for each summary bin, revealed water column stratification occupied by the sunfish. We found sunfish occupied well-mixed (53%), frontal regions (32%) and stratified waters (15% of the time), but there was no clear link between diving profile and water column stratification (Table 13). Lastly, individual sections determined by the SMW analysis were also examined at diel temperature occupancy and for each of the behavioural sections no significant differences were found in temperature use (K – S test, $p > 0.05$ respectively). The remaining 16 SMW sections are given in Figure S3.

Table 13 Summary of the diving profiles patterns found for PSAT tracked sunfish in relation to the water column structure ($n = 19$ sections were distinguished).

	nDVM (%)	rDVM (%)	Other (%)	Surface (%)
Stratified	12.5	0.0	0.0	33.3
Frontal	37.5	50.0	33.3	16.7
Mixed	50.0	50.0	66.7	50.0

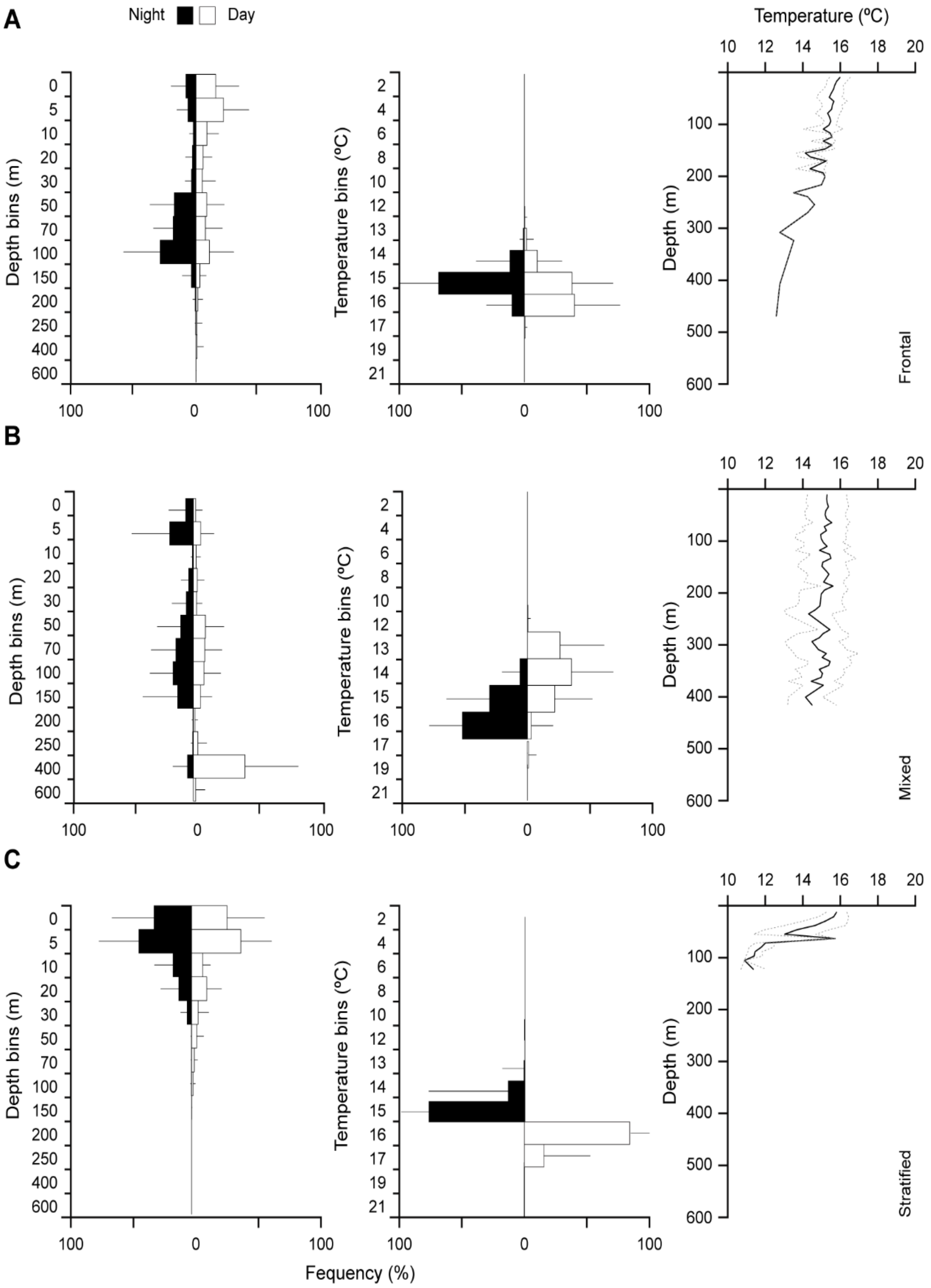

Figure 17 Example of tracked sunfish diving behavioural patterns and respective water column structure**.** Left panel shows the time at depth occupied by sunfish; central panel are the correspondent TAT profiles and panel in the right displays the water column structure, retrieved by the recorded depths and temperatures. All three marked diel behaviours are exemplified: A) reverse DVM in frontal waters; B) normal DVM in mixed waters; and C) surface oriented behaviour in stratified waters.

3.5 Discussion

Sunfish broad-scale movements were recorded for 18 satellite-tracked individuals and, significant size-based differences in the average daily movement distances and in the degree of apparent fidelity to an area were found in this study. Furthermore, we detected a seasonal trend in the sunfish dispersal, with individuals tagged in spring moving northwards whereas in winter a southern latitudinal preference was evident. We also detected focused residency areas, such as the Gulf of Cadiz, the north-west Iberian Peninsula and the Alboran Gyre. Importantly, these focal areas were identified after accounting for potential spatial bias of the tagging site on the number of satellite locations. Overall, sunfish movements in relation to changing environments were consistent with sunfish following thermal gradients (preferentially during summer months) and avoiding oligotrophic regions. Modelling sunfish habitat use, through RSPFs, confirmed that SST, thermal discontinuities and chlorophyll *a* statistically influence the sunfish probability of occurrence. The results of the influence of major currents on individual movements showed an ontogenetic rheotactic behaviour for sunfish; while small individuals often tend to swim with the major current's directionality, larger sunfish move more frequently against these mesoscale features. From the analysis of diving profiles of 9 individuals (≤ 1.00m) different vertical behaviours were found from nDVM, rDVM, surface-oriented and an irregular profile with no diel patterns. In addition, we also recorded shifts in diving behaviour with no apparent link with water column stratification. Taken together, these results, despite relatively few individuals, confirm satellite-based biotelemetry and oceanography as a powerful tool, providing valuable new insights into shifting habitat selection by ocean sunfish.

3.5.1 Distribution, horizontal movements and seasonality – environmental integration of sunfish movements

In this study, movement patterns of tracked sunfish provided further support that this species moves to higher latitudes in warmer months of late spring – summer in the north-east Atlantic

Ocean (Sims, Queiroz et al. 2009), similar to the pattern found in the north-western Atlantic (Potter, Galuardi et al. 2010). These north-south movements have been linked to the ability of the species to cope with seasonal variations in temperature (Sims, Queiroz et al. 2009) and were also observed in the Pacific Ocean. Sunfish satellite-tagged off Japan moved northward as waters warm (Dewar, Thys et al. 2010) and in the eastern Pacific a higher seasonal occurrence with the warming waters off California suggested a migratory activity also linked to SST (Cartamil and Lowe 2004). Furthermore, other marine predators, such as the blue shark, showed the same northward summer movements in the north-east Atlantic that are related to SST variations (Queiroz, Humphries et al. 2010), and the long-term satellite tracking of both the leatherback (McMahon and Hays 2006) and loggerhead turtles (Mansfield, Saba et al. 2009) also conform to this migratory pattern of higher latitudinal occurrence during summer periods. Similar to the leatherback turtle, sunfish also feed on gelatinous zooplankton, among other prey items (Pope, Hays et al. 2010), a feeding preference which may underpin the northwards movement as waters warm and zooplankton blooms in high latitudes (Parsons, Takahashi et al. 1984). These zooplankton blooms have been documented to attract higher-trophic level species such as sharks, bony fish, turtles and seabirds (e.g. Sims, Fox et al. 1997, Sims and Quayle 1998, Scales, Miller et al. 2014). Our results provide examples of individual sunfish northward movements apparently ‘riding a wave’ of optimal temperature envelope (increasing in summer) at higher latitudes (and SST gradients; Figure 3A). However, the relationship between northward extent and maximum primary productivity was less clear by comparison. The subsequent southward movements with local decreased temperatures, together with the southward movements observed in sunfish tagged in Ireland suggest a thermal niche for sunfish that does not extend to the lower temperatures characteristic of higher latitudes in winter, with sunfish tracked in this study not occupying waters with SST lower than 9º C. Interestingly, a clear size-related residency *versus* larger scale movements was found in the present study, with smaller sunfish remaining closer to the tagging region as opposed to larger individuals, which undertook wider displacements into more oceanic waters.

Space-use analysis indicated site fidelity to areas in the Gulf of Cadiz (GoC), the north-east Iberian Peninsula and the Alboran gyre. These patterns of temporal residency (having reduced tagging site bias) were in regions of seasonally enhanced productivity. The area off western Iberia is characterised by an equatorward upwelling jet during the upwelling season from May to October. This upwelling-driven frontal region is an important 'hotspot' for marine biodiversity (Chavez and Messié 2009, Sims, Queiroz et al. 2009, Walli, Teo et al. 2009), characterised by elevated bio aggregation, where the interface between offshore warmer oligotrophic waters with cool, nutrient-rich upwelled water has been shown to attract higher-trophic level foragers (e.g. Walli, Teo et al. 2009, Nur, Jahncke et al. 2011). Sunfish in this study were also found to spend longer periods in the vicinity of the Alboran gyre, within the Alboran Sea, which is known as the most productive area in the Mediterranean Sea (Siokou-Frangou, Christaki et al. 2010). Furthermore, the surface circulation in the GoC is directly linked to the north-eastern part of the North Atlantic subtropical gyre. A colder, open-ocean inflow in the region contrasts with the continental shelf warmer waters forming a frontal region (Stevenson 1977). In the eastern GoC the convergence of both Mediterranean and Atlantic waters enhance the primary productivity by strong thermal gradients (Relvas, Barton et al. 2007). Thus, although subject to seasonal and interannual fluctuations (Criado-Aldeanueva, García-Lafuente et al. 2009), this frontal area may be a major driver for the persistent occurrence of the smaller ocean sunfish we tracked in the region, mainly tagged during late spring and summer seasons. The GoC area is a well-known spawning and development region for other pelagic animals, including the European anchovy (*Engraulis encrasicolus)* (Catalán, Rubín et al. 2006), oceanic cephalopod paralarvae (Moreno, Dos Santos et al. 2009) and crustacean decapod species such as the rose shrimp (*Parapenaeus longirostris*) (Sobrino, García et al. 2007). Therefore, the elevated production in the coastal region of the GoC may be linked with a nursery area for younger stages of sunfish, by providing enhanced feeding opportunities that can support fast growth rates.

3.5.2 Sunfish habitat preferences

Although several studies have satellite tracked the horizontal movements of ocean sunfish (e.g. Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010, Potter, Galuardi et al. 2010), none has explored, in an analytical framework, the environmental factors driving this species' distribution and habitat selection patterns. Here we characterised the associations between sunfish and their immediate environment, using a presence *versus* available habitat design (Manly, McDonald et al. 2002). Our model of habitat selection revealed a spatial occupancy in the north-east Atlantic and in relation to water temperatures conforming to previous results in the region (Sims, Queiroz et al. 2009, Sims, Queiroz et al. 2009) and elsewhere (Dewar, Thys et al. 2010, Potter, Galuardi et al. 2010). Sunfish movements were related to a thermal envelope consistent with an 'escape' from maximum temperatures in the southern region in warmer months of spring and summer (≥ 25ºC, see Figure 9A). Likewise, the preference for frontal regions, also apparent in our model, has been described for other predators in the same geographical region as this study, linked to increased abundance of prey and thus, enhanced foraging opportunities (Sims and Quayle 1998, Pade, Queiroz et al. 2009, Queiroz, Humphries et al. 2012). Furthermore, our results confirm previous observations in the north-east Atlantic (Sims and Southall 2002) and more recently in the Pacific, where sunfish were associated with upwelling frontal regions (Thys, Ryan et al. 2015). Animal movements are expected to be driven by environmental suitability to satisfy the species intrinsic demands, such as feeding, which in turn is motivated by the abundance of prey (e.g. Hays, Hobson et al. 2006, Sims, Witt et al. 2006). Hence, despite not being significant the negative influence of chlorophyll *a* in the overall sunfish habitat selection is a somewhat counterintuitive result, given the expectation of increased occurrence of predators in highly productive regions. One possible explanation for this is that sunfish do not track primary productivity directly, but rather move northwards as preferred thermal conditions extend latitudinally and coincide with zooplankton blooms that then occur seasonally. Importantly, with the exception of a few months, a temporal mismatch between primary production and zooplankton was detected in the monthly patterns of phytoplankton and total copepod abundances (zooplankton) for the Goban Spur region, North Atlantic (Sims, Southall et al. 2003).

Lastly, the modest positive influence of SST anomaly in the overall habitat selection of sunfish may reflect longer term trends in migratory patterns of the species.

Sunfish seasonal occupancy in the area predicted by the logistic RSPF is consistent with the inference from our satellite tracked movements, thus showing the utility of the model for making accurate broad-scale sunfish habitat use estimates. The modelled habitat distribution also identified probable high use areas not visited by tracked sunfish. For example the sunfish 'hotspot' predicted off southwest England (Figure 5C) has been validated by ship and aerial surveys with high sunfish abundances reported in previous studies (Sims and Southall 2002, Leeney, Witt et al. 2011). This emphasises the value of understanding how sunfish use habitats across their distributional range, which. Understanding the relation between sunfish and the environment within a model allows the determination of potential habitats, ultimately improving our ability to predict future shifts in the population distribution due to environmental changes (Boyce, Vernier et al. 2002).

In this study, the seasonal sunfish movement towards northern latitudes during summer was correctly estimated by the model (Figure 5C), and may be explained by the avoidance of high temperatures at southern latitudes during warmer months. During summer, the increased water temperatures in the region might drive sunfish northward, with the fish possibly moving away from excessive temperatures while also tracking the intense frontal region off western Iberia, which in summer is characterised by a strong upwelling phenomena approximated by significant positive SST gradients in the model. In winter, the model correctly estimated the southern movements of sunfish (Figure 5E), which conform to the suggested thermal tolerance for this species (above the minimum of 9.9º C) (Sims, Queiroz et al. 2009). Similarly, the identified southern movement of sunfish during cooler months, either to warmer Mediterranean waters or along the northwest African upwelling region, revealed a good approximation of our model to the broad scale distribution of sunfish in the region. The negative influence of primary productivity found in our overall model was maintained during summer, spring and autumn months. Hence, the preference for other environmental conditions, such as the strong thermal gradients representing frontal regions, may be of more importance for the species distribution than primary productivity *per se.* Importantly, sunfish likely feed on organisms that consume primary productivity (e.g. phytoplankton) and thus the negative chlorophyll *a* estimate likely reflect the presence of sunfish prey.

In summary, the overall predictive map of sunfish habitat selection generates probable habitats for sunfish across much broader scales than was possible with the obtained tracking locations reported in this study, but which seem consistent with incidental reports of sunfish hotspots (Sims and Southall 2002, Leeney, Witt et al. 2011). These results with relatively few individuals, confirm the usefulness of the integration of satellite-based biotelemetry with oceanography in improving our knowledge into shifting habitat selection by a marine predator. The methodology employed to account for the different tracking systems (Argos and light-level) spatial inaccuracies ensured the incorporation of a more complete tracking dataset covering all seasonal periods, and thus informed the spatial dynamics and environment-use of the ocean sunfish.

3.5.3 Currents influence on sunfish movements

Our results largely support the findings that sunfish perform consistent directed movements independent of the direction of oceanic currents (Cartamil and Lowe 2004, Watanabe and Sato 2008, Sims, Queiroz et al. 2009, Sims, Queiroz et al. 2009). Rheotaxis (positive – facing upstream) is an important and general behaviour in fish (Montgomery, Baker et al. 1997, Baker and Montgomery 1999) which assist the individual to maintain the position at a minimum cost, detect chemical cues, prey or predators and/or to migrate (Peach 2001). In this study however, we detected a size dependent rheotactic behaviour in tracked sunfish. Whereas larger fish were found to display more often a positive rheotaxis, smaller individuals did not swim frequently against the direction of major currents. Our results conform to, for instance, the seasonal occurrence of small sunfish off Plymouth UK, which were suggested to be carried further inshore than adults, due to water movements (Sims and Southall 2002).

Different explanations could be advanced to explain the size dependent rheotactic behaviour in sunfish. Firstly, the size-related residency *versus* dispersed patterns could be driven by reproduction purposes. Maturation is frequently advanced as a possible explanation for partial migration behaviours, where only a portion of the population migrates (Boyle 2008, Chapman, Hulthén et al. 2012, Papastamatiou, Meyer et al. 2013). However, sunfish maturity is assumed to be attained at 1.40 m TL (Nakatsubo, Kawacxt et al. 2007), a size not reached by the larger individuals tracked in this study. Moreover, the long distance travelled by one immature fish of 0.60 m does not support reproduction as a driver for the observed disparity in the spatial occupancy. Even though our sample size may not be large enough to identify this pattern, individual growth and body condition, with inherent energetic demands, are possibly driving the described patterns. Secondly, the energetic costs associated with larger scale movements in the highly dynamic marine environment may account for the higher frequency of residency observed in smaller fish. Furthermore, we suggest that diet preferences may be underpinning the observed different patterns in relation to size. Recent studies based on isotopic signatures in sunfish tissues have empirically contradicted the obligate gelatinous diet of sunfish (Syvaranta, Harrod et al. 2012, Harrod, Syvaranta et al. 2013, Nakamura and Sato 2014), revealing that smaller fish likely feed on benthic coastal prey. These studies suggest that smaller individuals have a preference for mixed prey type, with isotope signatures of small fish revealing the consumption of prey species other than gelatinous zooplankton, which is likely the preferred prey of larger sunfish. Moreover, sunfish swimming stroke and foraging behaviour has also been directly related to size, reflecting an ontogenetic shift in behaviour linked to feeding and presumably dietary preferences (Nakamura and Sato 2014). More recently and through animal-borne cameras deployed off Japan, larger sunfish were found feeding on gelatinous species at depths (Nakamura, Goto et al. 2015). Hence, it is possible that sunfish positive rheotactic behaviour observed in larger individuals may be a response to an odour stimulus, where individuals swim up-current towards an odour source such as large gelatinous prey.

Positive rheotaxis coupled with broader scale movements suggest that larger sunfish opportunistically take advantage of migrant prey; and that smaller fish, with limited spatial movement and predominant negative rheotaxis, persist in higher prey abundance areas. By swimming more often against the currents directionality, larger sunfish likely have higher prey encounter rates, since smaller zooplankton species are often transported by major horizontal currents, with the exception of jellyfish which have been found to perform active flow-oriented movements (Fossette, Gleiss et al. 2015). On the contrary, smaller individuals which tend to remain in coastal waters take advantage of prey entrapped by major oceanographic currents in the GoC. Hence, it is likely that circulation patterns and high productivity in the GoC (Relvas, Barton et al. 2007, Torres, Coll et al. 2013) dictate the residency or migratory behaviour of sunfish that also depend on body condition and/or maturation state.

3.5.4 Diving profiles of sunfish

Vertical diving records from ocean sunfish revealed a consistent broad range of depths in all nine individuals, with sunfish occupying the water column from the surface to 704 m (480 ± 125 m). This vertical range varied with tracked individuals' size, even though they did not exceed 1.00m TL. The larger individuals displayed significantly extended depth amplitudes and increased time in deeper layers (>250m), which may reflect sunfish tracking vertically migrating prey (Houghton, Doyle et al. 2008; Sims, Queiroz et al. 2009). Importantly, current shear will disperse the scents and tastes (Carey and Scharold 1990) and sunfish will likely have a greater chance of encountering a prey/food trail by moving vertically through the layers. Moreover, the recent fine-scale tracking of sunfish off Japan, lead the authors to suggest that large body mass enhances the ability of the species to cope with vertical temperature gradients (Nakamura, Goto et al. 2015).Our results, showing greater amounts of time in deep water for larger fish are consistent with this observation.

Overall, our analysis confirmed the predominance of an overall nDVM behaviour in sunfish (Cartamil and Lowe 2004, Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010). However, several patterns were detected and our study suggests that in the north-east Atlantic sunfish of smaller sizes (≤1.00m) exhibit different diel depth preferences, with the first indication of a reverse DVM being presented. Sunfish are known for significant deep dives and it was suggested that surface times would be a mechanism to compensate the heat lost at colder waters in depth (Cartamil and Lowe 2004). This was recently confirmed in a study with sunfish tagged off Japan, where the authors described a thermoregulatory behaviour occurring at a scale of several minutes (Nakamura, Goto et al. 2015). In our study however, the coarse resolution of our depth data makes a similar investigation impossible. Further tracking of sunfish providing high-resolution dive profiles (minutes), would be required to identify such finer scale behaviours in this region (Nakamura, Goto et al. 2015). Importantly though, our results show that the depths sunfish occupy during the day and night do not vary geographically, with all vertical behavioural patterns (at different times) occurring in the same area. Strikingly, SMW identified significant differences in sunfish behaviours with no linkage to a specific water column structure. All four behavioural modes (nDVM, rDVM, surface oriented and other) were found both in frontal and well mixed waters, and when in stratified waters sunfish either performed nDVM or surface-oriented patterns (Table 4). Hence, although significant changes in the vertical behaviour of sunfish were found, there was no clear link between fish behaviour and the thermal structure of the water column.

Sunfish DVM has also been proposed to be a foraging strategy (Cartamil and Lowe 2004, Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010), with fish potentially tracking DVM of gelatinous prey in the water column (Houghton, Doyle et al., 2008, Hays, Bastian et al. 2012). Similarly, the archival tracking of the plankton-feeding basking shark together with zooplankton monitoring with nets and echosounders demonstrated that this shark undertakes rDVM in frontal habitats that are dominated by *Calanus* copepods exhibiting rDVM to avoid predatory invertebrates (chaetognaths, arrow worms) (Sims, Southall et al. 2005, Shepard, Ahmed et al.

2006), whereas in stratified waters they exhibit nDVM (Shepard, Ahmed et al. 2006). In this study, sunfish rDVM was performed equally when in frontal regions and well-mixed waters, whereas nDVM and surface-oriented behaviours occurred in all three water column structure types. Another gelatinous predator, the leatherback turtle, was found to display daily deep diving in some areas, and shallow diving with no diel patterns in others, probably mirroring prey behaviour (Hays, Hobson et al. 2006, Houghton, Doyle et al., 2008). High-resolution vertical profiles of jellyfish revealed, however, the lack of a systematic day–night shift in depth (Hays, Bastian et al. 2012). Furthermore, stable isotope analysis suggests an ontogenetic shift in the dietary habits of sunfish off Japan (Nakamura and Sato 2014); with smaller sunfish focusing within coastal food webs, whilst larger individuals mainly feed on vertically migrating prey in deeper waters. Here, sunfish tracked with PSAT depth tags were small (≤1.00m) and for individuals this size, prey preferences are less clear. Yet, it seems likely that a combination of both benthic crustaceans and gelatinous, vertically migrating species may be consumed by these sizes of fish (Nakamura and Sato 2014). Hence, the highly variable diving patterns recorded in our study confirm these assumptions, further supporting the broad feeding habits of smaller sunfish. Altogether, these results suggest a widespread behavioural variability in the diving patterns of ocean sunfish, similar to several other generalist predators, and probably in response to diverse prey distributions and/or behaviours.

3.6 Conclusions

In summary, by satellite-tracking 18 individuals in the north-east Atlantic a broad range of vertical and horizontal movement patterns in sunfish was revealed. Seasonal movements were identified, with sunfish favouring northern latitudes in warmer months, whereas in colder periods the species tended to reside at more southern locations. Despite these clear seasonal patterns, longer tag attachments providing yearly recordings for this species are desirable to confirm migratory behaviour. In addition, spatial focal residency in highly productive regions was also reported, with track-estimated habitat selection being driven by SST and highly influenced by thermal gradients.

We also detected a distinct displacement and rheotactic behaviour related to sunfish size, with sunfish increased tendency to a positive rheotaxis with growth, which may be explained by the ontogenic shift in preferred prey intake from benthic crustacean to gelatinous zooplankton prey. Going with currents is obviously advantageous in travelling movements, whereas the benefit of counter-advection by the currents when feeding on advected prey should also be considered as part of the sunfish's foraging strategy. Moreover, four different diving behaviours were recorded indicating similarities between sunfish and other marine vertebrates. Observed patterns are probably associated with prey behavioural differences as no geographical or water column stratification dependencies were found. Moreover, even though no adult sunfish (larger than 1.4m Nakatsubo, Kawacxt et al. 2007) were tagged in this study, we have identified a size-specific pattern in both horizontal and vertical movements. We found larger sunfish travelled longer distances while exploring increased depth amplitudes, where they stayed for longer, compared to smaller individuals. From previous studies, sunfish was found to have different trophic associations in relation to fish size (Syvaranta, Harrod et al. 2012, Nakamura and Sato 2014) and our results are consistent with divergent prey preferences and feeding strategies.

Chapter 4: Dietary habits of ocean sunfish revealed through DNA barcoding

4.1 Abstract

Conservation and management measures of both marine species and ecosystems require a better understanding of the mechanisms underlying predator-prey interactions. Although for a long time the ocean sunfish (*Mola mola)* was thought to feed exclusively on gelatinous prey, recent studies suggest that it may be a more generalist predator. However, previous work were very informative on the trophic level clarification, no empirical assessment of the diet contents of sunfish of smaller sizes was performed, to our knowledge. In this study, we used a molecular barcoding approach to characterize the dietary preferences of sunfish caught from the Gulf of Cadiz off southern Portugal. Altogether, we were able to identify a total of 41 different prey items, of which 32 were identified down to species level, four to genus, two to family, one to infraclass and two to class. Overall, sunfish were found to feed mainly on crustaceans and teleost fish, with cnidarians (the long suggested preferred prey) comprising only 16% of consumed prey items. Although no

adult fishes were sampled in this study, we found evidence for an ontogenetic shift in sunfish dietary preferences, with smaller individuals [<0.80m] feeding mainly on small crustaceans and teleosts whereas the diet of larger fish [>0.80m] included cnidarian species. Therefore, our results support the view that smaller sunfish feed predominantly on benthic prey and to a lesser extent on prey in coastal pelagic habitat, whereas larger sunfish depend to a much greater degree on prey within the water column. These findings confirm using genetic approaches that the ocean sunfish is a generalist predator with greater links throughout the coastal marine food web than previously realised. Therefore, its removal as bycatch may have ecological consequences, potentially disrupting trophic interactions and ecosystem functioning especially in its coastal nursery areas.

4.2 Background

An important constraint in ecological studies, especially in the aquatic marine environment, is the lack of adequate knowledge regarding trophic interactions (Corse, Costedoat et al. 2010, Jo, Gim et al. 2014). While direct observations of feeding events and examination of either gut or faecal contents have provided important information regarding marine food webs, these data are still scarce (Biuw, Boehme et al. 2007, Bestley, Patterson et al. 2010). Moreover, analyses of stomach contents imply invasive sampling procedures such as stomach flushing or, require examination of dead animals (Jarman, Gales et al. 2002, Parsons, Piertney et al. 2005, Dunshea 2009, Bucklin, Steinke et al. 2011). Such analyses rely on the correct identification of individual prey items which can be difficult after several hours of digestion (King, Read et al. 2008). Importantly, while undigested individual prey items may be easily identified through morphology, consumed items often consist of a pool of indistinguishable prey remains (Passmore, Jarman et al. 2006, Ahrens, Monaghan et al. 2007) or morphologically similar prey (Barrett, Camphuysen et al. 2007). Taking advantage of the global barcoding efforts (Savolainen, Cowan et al. 2005) which have resulted in substantial public databases containing DNA information from thousands of organisms, target amplification of the so-called barcoding genes becomes a viable option to identify digested and partially digested prey items recovered from animal stomachs (Valentini, Pompanon et al. 2009, Jo, Gim et al. 2014).

The application of DNA barcoding in dietary studies of large, predatory fish that are difficult to observe directly will likely help to resolve prey interactions and how these may change during ontogeny. The ocean sunfish is a widely distributed marine predatory fish but little is known about its ecology and, until recently, it was regarded as an obligate gelatinous zooplankton feeder (Pope, Hays et al. 2010). This assumption, however, has only been supported by indirect evidence, such as the distribution of fatty acids in the lipids found in four sunfish individuals caught off Nova Scotia (Hooper, Paradis et al. 1973), and the co-occurrence of ocean sunfish and three jellyfish species (*Rhizostoma octopus, Chrysaora hysoscella* and *Cyanea capillata*) in the Irish and Celtic Seas (Houghton, Doyle et al. 2006). Recent stable isotope analysis (SIA) of putative prey and muscle tissues from the dorsum of 8 sunfish captured in the Mediterranean, challenged this view, revealing the possibility of different prey preferences and/or the occurrence of an ontogenic shift in the diet (Syvaranta, Harrod et al. 2012). The conclusions of this work raised some debate (Harrod, Syvaranta et al. 2013, Logan and Dodge 2013), with the latter authors suggesting that the data were insufficient to disprove that sunfish are obligate consumers of gelatinous organisms (Logan and Dodge 2013). Nevertheless, results from the recent work of Nakamura and Sato (2014), coupled with SIA with the visual identification of stomach contents from 17 sunfish captured off Japan, also suggest an ontogenetic shift in diet with age, with smaller fish feeding on benthic crustaceans, whereas larger individuals feed on gelatinous animals occurring in the water column. This preference in larger individuals was also confirmed by visual (animal-borne camera) recordings of feeding events on siphonophores, syphozoa and ctenophores (Nakamura, Goto et al. 2015). In summary, the only conclusive diet data for sunfish in the Mediterranean relies on isotopic similarity to more neritic fishes; the video-recording of gelata consumption by larger individuals and the presence of crustaceans in stomach contents of sunfish of sizes smaller than 0.5m off Japan. Hence, the lack of a comprehensive study on the diet components of sunfish in the north-east Atlantic prompt this work.

In recent years, the emergence of next generation sequencing (NGS) has provided a powerful new approach to complex dietary studies (Pompanon, Deagle et al. 2012). This technology is responsible for an increasing number of studies using molecular barcodes to reveal marine species diets such as coral reef fish (Leray, Yang et al. 2013), harbour seals (*Phoca vitulina*) (Deagle, Thomas et al. 2013, De Barba, Miquel et al. 2014), Adélie penguins (*Pygoscelis adeliae*), the endangered pygmy blue whale (*Balaenoptera musculus brevicauda*) (Jarman, Gales et al. 2002) and little penguins (*Eudyptula minor*) (Deagle, Chiaradia et al. 2010). The implementation of such a barcoding approach is however, far from straightforward (Passmore, Jarman et al. 2006, Ahrens, Monaghan et al. 2007, Vestheim and Jarman 2008, Valentini, Pompanon et al. 2009, Bucklin, Steinke et al. 2011, O'Rorke, Lavery et al. 2012, Leray, Yang et al. 2013). While universal sets of primers are often used to maximize DNA detection from the widest possible range of prey species, they often enable the predator’s DNA to be co-amplified. As predator DNA is highly prevalent in gut contents it can be preferentially amplified over prey thus hampering barcoding efforts (Leray, Yang et al. 2013). Thus, predator DNA removal techniques are highly recommended in diet studies using molecular barcodes (Blankenship and Yayanos 2005, O'Rorke, Lavery et al. 2012). Different approaches are currently available to eliminate the confounding predator DNA. One such technique is the use of commercially available restriction enzymes that will specifically target host amplicons (Blankenship and Yayanos 2005, Blankenship and Levin 2007). Although this technique has been successfully applied to identify prey items of some marine mammals (Parsons, Piertney et al. 2005, Dunshea 2009), it requires a unique endonuclease cutting site present in the predator sequence, which is often difficult to find (Vestheim and Jarman 2008). Moreover, if host DNA is prevalent, there is a chance that DNA amplification is biased towards the predator in the first place, thus still impeding prey detection. A cost-effective alternative to restriction enzymes is the use of predator specific non-extendable primers - the *blocking* primers, during the DNA amplification procedure. These specific primers are designed to overlap with the universal primers and to bind with the predator DNA, preventing its amplification (Vestheim and Jarman 2008, Leray, Yang et al. 2013).

Taking advantage of such improvements in the DNA barcoding techniques, in this study we developed a strategy where universal PCR and predator-specific *blocking* primers were simultaneously used to amplify barcode genes from the stomach contents of the ocean sunfish. Here, the main objective was to determine at the highest resolution possible (ideally at the species level), the prey preferences of the sunfish in the north-east Atlantic and determine its trophic position in food webs. By doing so, we tested the generalist predation and ontogenetic shift in the diet of sunfish, advanced by the stable isotope studies.

4.3 Methods

4.3.1 Sunfish stomach contents collection

We collected gut contents from a 100 individuals found dead in a set-net targeting tuna, off Olhão, southern Portugal. Here, dozens of healthy sunfish are captured and released daily. Occasionally, however, due to adverse weather conditions, daily fishing operations are disrupted which may result in the death of some trapped fish. Our sampling targeted such dead fish and occurred during April – June (spring) months of 2013 and 2014, with 50 individuals being sampled each year. Digestive tracts were collected during fishing operations, maintained on ice while on-board (< 1 hour) and immediately stored at -20ºC. In the laboratory, undigested remainders from gut contents were morphologically identified and photographic recorded through a dissecting microscope.

4.3.2 DNA Extraction and amplification

Gut contents mostly consisted of a homogenate from which genomic DNA was extracted using the JetQuick™ DNA Kit (GENOMED®), following the protocol for purification of total DNA from animal tissues, with some adaptations. The first change to the standard protocol was the volume of reagents used to digest the stomach content: a total of 4 ml of lysis buffer was added to 2 ml of gut content of each individual, along with 60 µl of proteinase K, in a 15 ml tube; the samples were incubated overnight at 56ºC. Due to the initial large volume, several repetitions of the following steps of the protocol had to be performed. In addition, DNA from sunfish muscle and that of the pelagic crab, *Polybius henslowii*, which was frequently observed in the gut contents, was also extracted, following the standard protocol, and were used as controls for the subsequent polymerase chain reactions (PCR). Extracted DNA was immediately frozen at -20ºC until PCR was carried out.

We used degenerate versions of the universal COI (cytochrome oxidase subunit I) primers LCO1490 and HCO2198 (Folmer, Black et al. 1994), the jgLCO1490 (5'TITCIACIAAYCAYAARGAYATTGG 3') and the jgHCO2198 (5'TAIACYTCIGGRTGICCRAARAAYCA 3') which are recommended for metabarcoding studies (Geller, Meyer et al. 2013). To quantify the amplification of sunfish DNA, PCR was performed for a random subset of 10 individuals and sequencing through cloning (10 clones per individual). Since over 60% of the sequences retrieved belonged to the sunfish, we opted to design a blocking primer 5'- CAAAGAATCAGAAGAGATGTTGA [SpcC3]-3' based on the mitochondrial genome of sunfish available on GenBank (Accession number AP006238). This primer overlapped with the 3' end of the reverse universal primer, but extended into the sunfish specific sequence, and was modified with a C spacer (following Vestheim, H et al., 2008), to prevent elongation without affecting the annealing properties. To investigate possible diet shifts with sunfish size, sampled individuals were grouped into four different fish size classes (< 0.40 m; 0.40 - 0.60 m; 0.60 - 0.80 m and > 0.80 m TL), which encompassed all the individuals for which stomach contents were available and maximised, in terms of costs, the identification of a specific size at which sunfish changed their diets. Additionally, in PCRs to be sequenced through NGS, we used four different multiplex identifiers from Roche^{TM} (MIDs) attached to the original primer sequences (MID001: ACGAGTGCGT; MID002 ACGCTCGACA; MID003: AGACGCACTC; MID004: AGCACTGTAG).

For DNA amplification, 1 µl of the extracted DNA was added to a total of 19 µl of PCR reaction mix and proof reading Platinum Taq^{TM} (Invitrogen^{TM}) was employed in all PCR reactions (see Table 14 for PCR mix descriptions). Firstly, we tested the efficiency of two different concentrations of blocking primer [10 and 20 x] relative to the universal COI primers, to prevent sunfish DNA amplification during PCR. We found that a proportion of 20:1 of blocking primer was needed to ensure minimum sunfish DNA amplification.

A touchdown PCR amplification protocol was implemented due to the higher temperature demand of the blocking primer to bind to the sunfish DNA. Thermal cycling conditions were as follows: initial denaturation at 95°C for 5 minutes followed by 62 cycles of: 95°C for 30 seconds; annealing temperature step-downs every 4 cycles of 1°C (from 55°C to 47°C) until the temperature reached 46ºC which was maintained for 20 cycles; 72°C for 30 seconds; and a final extension at 72ºC for 7 minutes. DNA amplification of the stomach contents and *P. henslowii*, and absence of amplification for the sunfish DNA and PCR reagents negative control (blank) were verified in an agarose gel (e.g. Figure 18). PCR reactions used in NGS were repeated at least three times per sample, and were pooled as explained in the next section.

Table 14 PCR reaction mix per individual (20 µl).

	Cloning	**NGS**
$H_2$0	12.25	11.80
PCR Buffer	2.08	2.00
$MgCl_2$ (50mM)	1.04	1.30
dNTP mix	0.41	0.40
Primer jgLCO1490	0.82	0.80
Primer jgHCO2198	0.82	0.80
Blocking primer	1.63	1.60
Taq Polymerase	0.16	0.20
Bovine Serum Albumin	0.10	0.10

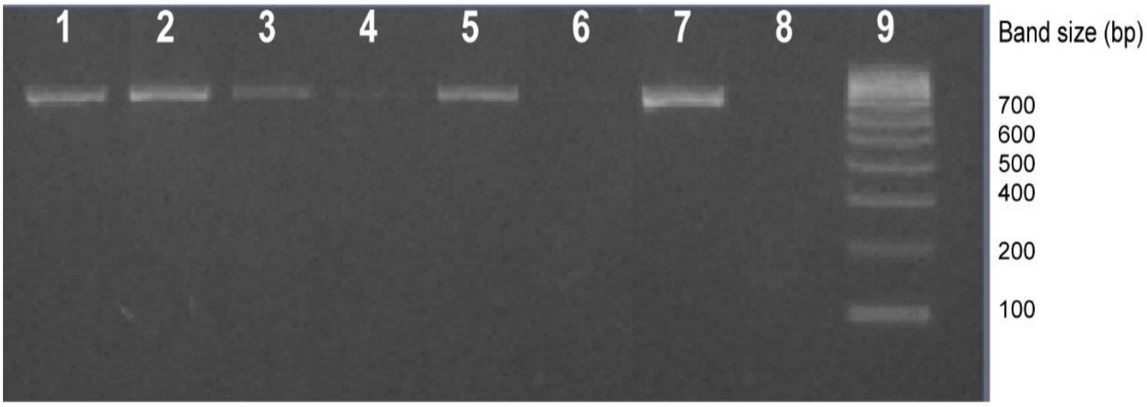

Figure 18 PCR confirmation of the efficiency of ocean sunfish specific blocking primer. Gel lanes correspond to: prey item amplified DNA (1-5); sunfish DNA (6); P. henslowii DNA – positive control (7); reaction negative control (blank) (8) and molecular ladder (NZYDNA ladder V) (9).

4.3.2.1.1 Cloning and NGS

A total of 38 PCRs were sent to an external service for cloning (Centro de Testagem Molecular - CTM; CIBIO InBIO-UP) and on average 10 clones per sample were sequenced. For the 96 PCRs prepared for NGS, purification was accomplished using AGENCOURT® AMPURE® XP following the manufacturers' protocol. Final purified PCR product was eluted in 28 µl of water. DNA quantification was performed using Quant-iT™ PicoGreen® dsDNA Assay Kit. Samples for which DNA concentration did not exceed the 10 ng/µl, the minimum requested for NGS proceeding, were concentrated using Speed Vac Eppendorff at room temperature, during cycles of 45 minutes, until a final volume of ca. 13 µl. Finally, PCR products were pooled and sent to Beckman Coulter Genomics® to be sequenced by a Roche 454 GS FLX Sequencing Platform.

4.3.3 Data treatment

4.3.3.1 Identification of sequences retrieved through cloning

We performed Basic Local Alignment Search Tool BLAST - searches against sequences present in the NCBI GenBank[3] and BOLD[4] databases. All possible genetic codes (Invertebrate; Vertebrate; Echinoderms; Ascidian) were investigated to ensure the best translation possible. Additionally, sequences were translated into protein and protein BLAST searches were performed to discard pseudo genes. Resultant identification at species level was only accepted if the sequence similarity with best match exceeded the 97% (Altschul, Gish et al. 1990).

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[3] http://www.ncbi.nlm.nih.gov/

[4] http://www.boldsystems.org/index.php/

4.3.3.1.1 Metabarcoding (NGS) sequences treatment: Filtering, alignment, clustering and Taxonomic assignment

We obtained a 454 platform output “standard flowgram file” demultiplexed by MID (sunfish size class). Next, a Pyronoise procedure was implemented using the *mothur* software package version 1.35.1 (Schloss, Westcott et al. 2009) and following the standard operating procedure (SOP) to process sequences generated by 454 pyrosequencing[5]. Briefly, only sequences longer than 300bp and unique haplotypes were retained for further analysis. Additionally, whenever more than 8 homopolymers (likely sequencing errors) were present in sequences these were discarded. Subsequently, following Ranwez et al. (2011), Macse v1.01b was then implemented to translate automatically nucleotide into amino acid sequences and perform BLAST searches within GenBank, using all possible genetic codes. A twofold downstream analysis was performed: (a) nucleotide sequences were blasted to firstly remove sequences with stop codons [bacteria or pseudo genes], chimeric sequences and frame shifts [presumably caused by errors during the 454 platform sequencing procedure (Leray, Yang et al. 2013)], together with sequences with similarity of 97% or lower (Altschul, Gish et al. 1990, Leray, Yang et al. 2013); (b) amino acid translated sequences were also taxonomically assigned to the lowest taxonomic group according to the homology attained per sequence. Further detailed description of the methodology employed can be found in the supplementary material. We found this approach useful to detect errors in the nucleotide Genbank database, namely bacterial sequences mislabelled as fish and other marine organisms (Siddall, Fontanella et al. 2009). Furthermore, we also used the BOLD database to compare our sequences and further confirm the results with GenBank.

Lastly, a Bayesian approach implemented in the Statistical Assignment Package (SAP) (Munch, Boomsma et al. 2008) that uses the Genbank reference database, was employed to

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[5] http://www.mothur.org/wiki/454_SOP

assign those sequences with lower similarity scores (< 98%) to the highest taxonomic rank possible. Briefly, the posterior probability of a nucleotide sequence belonging to a specific taxonomic rank represented in Genbank database was calculated by building 10,000 phylogenetic trees. To do so, a total of 50 homologue sequences with similarity scores higher than 70%, were downloaded from Genbank and taxonomic assignments were only made at significance level equal or higher than 95% (Munch, Boomsma et al. 2008). Further details may be found in the supplementary material.

4.3.3.1.2 Prey identification per sunfish size class: Quantifying diet composition and overlap

To investigate the differences in diet composition and in relation to sunfish size, sampled individuals were grouped into classes of sizes and overlap among the four different size classes we measured the prey variability among each class using the Bray–Curtis dissimilarity index implemented in the ‘vegan’ package in R software. This rank-order similarity index was then applied to prey taxa grouped by sunfish size class.

4.4 Results

4.4.1 Sizes distribution of captured sunfish

Total length of sampled individuals ranged from 0.37 to 1.10 m, corresponding to juveniles and sub-adults (Nakatsubo, Kawacxt et al. 2007). The size distribution of captured fish is shown in Figure 18. This distribution also reflects the total length (TL) distribution of sunfish captured in the set-net (*personal observation*); with the majority of captured sunfish ranging from 0.40 – 0.60 m, with larger fish being less frequent.

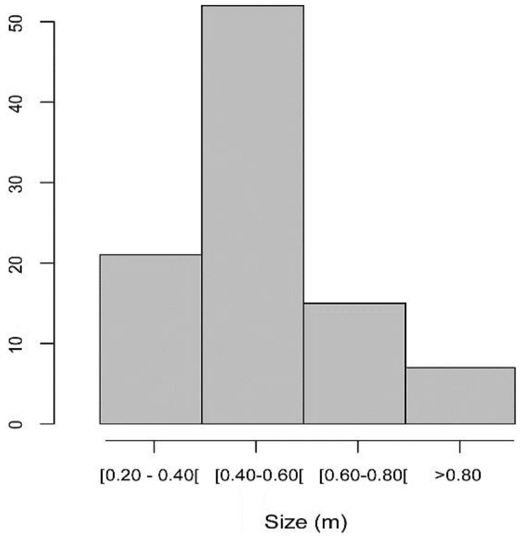

Figure 19 Individual sunfish sizes classes from which digestive tracts were collected.

4.4.2 Prey morphological identification

Besides the frequent visual identification of the swimming crab *Polybius henslowii* appendages, further undigested prey items recovered from stomach contents comprised other crustaceans (e.g. Gammaridae, Corophiidae and a megalopa larvae) and a *Conger sp*. larvae (see Figure 20). In this study, undigested prey or body parts were found only in fish of sizes < 0.60 m (size classes 1 and 2). In addition, cestode parasites were found in 63 of the 100 sunfish intestines.

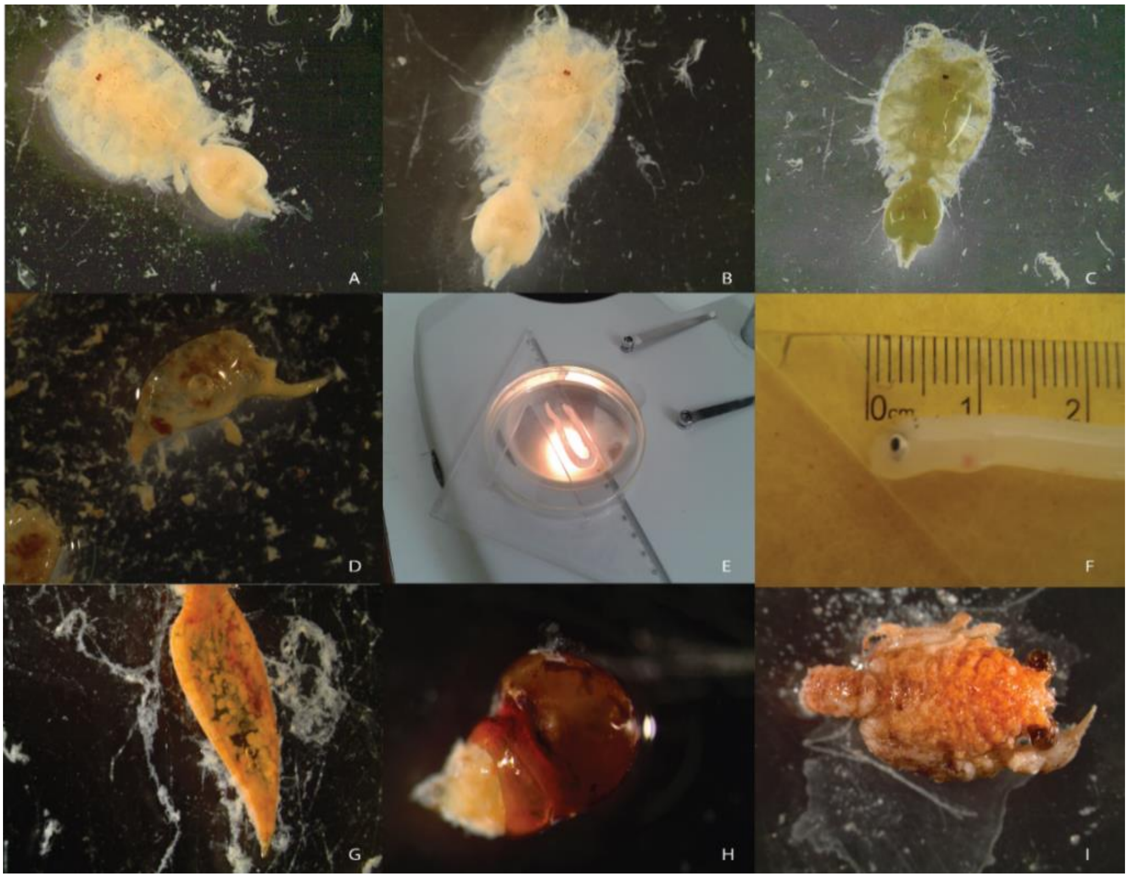

Figure 20 Visual identification of undigested remains within stomach contents of sunfish. A-C marine ectoparasites (Caligidae); D unidentified crustacean appendage (cheliped); E-F eel larvae (11 cm); G-H appendages of an unidentified crustacean; I Megalopa crustacean larvae.

4.4.3 Overview of sequencing data

We successfully amplified DNA from the stomachs of 57 sunfish. All PCRs were sent for cloning and from these a total of 33 were successfully sequenced (totalling 119 clones). Despite this effort, only eight unique prey items were identified to species level (Table 15). A total of 5 clones were identified as sunfish, making up less than 5% of the total sequences. After obtaining these results, a second batch of PCRs were performed for NGS (approximately 8 months later), and a total of 36 samples were successfully amplified. We presume that the decrease in amplification success was due to deterioration of prey DNA after consecutive freezing and defrosting. A total of 75,091 DNA sequences were retrieved from the sequencing platform. Of these, 4152 (~5%) were identified in an initial blast as marine bacteria and were removed from the dataset. The final eukaryotic dataset comprised 70,939 sequences, of which sunfish made up a total of 3355 amplicons generated (less than 5%). A total of 39 unique prey were detected, of which six were also detected using cloning (**Error! Reference source not found.**). Lastly, from the 39 prey items ecovered via NGS, 14 were only retrieved after SAP processing.

4.4.4 Sunfish prey items recovered

A total of 41 single prey items were identified from the stomach contents of sampled sunfish, belonging to eight different taxonomic classes (Figure 21). Of these, 27 were found at the similarity level of ≥ 98% using nucleotide sequence blast in GenBank and BOLD databases, and a further 14 prey items were subsequently found at 95% probability through Bayesian statistics in SAP. In total we found species from 5 Phyla and 8 Classes. Overall, Malacostraca comprised ~37% of the identified sunfish diet, Actinopterygii (24%), followed by Hydrozoans at 15%. The remaining ~24% were Maxillopoda, Bivalvia, Cephalopoda and Gastropoda (Figure 21).

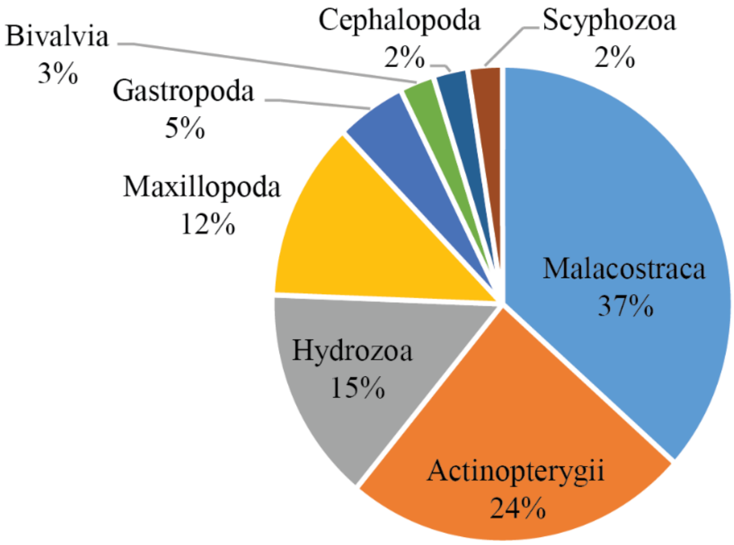

Figure 21 Summary of the prey taxa identified from sunfish stomach contents.

Table 15 List of individual prey items identified through barcoding, with accession number from GenBank database, Taxonomic Class and sequencing method.

Size Class	**Accession**	**Phyla**	**Class**	***Lowest taxonomic level***	**Method**	**Similarity**	**Bayesian probability**
1, 2 and 3	HQ167651	Chordata	Actinopterygii	*Hygophum benoiti*	NGS	≥98%	-
1, 2 and 4	EF989670	Arthropoda	Malacostraca	*Phrosina semilunata*	NGS/cloning	≥98%	-
1, 3 and 4	GQ120035	Cnidaria	Hydrozoa	*Physophora hydrostatica*	NGS/cloning	≥98%	-
1 and 2	JQ306133	Arthropoda	Malacostraca	*Funchalia villosa*	NGS	≥98%	-
1, 2 and 3	AY861363	Arthropoda	Maxillopoda	*Lepeophtheirus pollachius*	NGS/cloning	≥98%	-
1 and 3	JQ775007	Chordata	Actinopterygii	*Conger conger*	NGS/cloning	≥98%	-
1 and 3	JQ306263	Arthropoda	Malacostraca	*Pasiphaea sivado*	NGS	≥98%	-
1 and 3	*	Arthropoda	Maxillopoda	*Caligus (genus)*	NGS	≥70%	≥95%
1 and 3	*	Chordata	Actinopterygii	*Teleostei (infraclass)*	NGS	≥70%	≥95%
2 and 3	*	Cnidaria	Hydrozoa	-	NGS	≥70%	≥95%
2 and 3	GQ268538	Arthropoda	Malacostraca	*Liocarcinus holsatus*	NGS	≥98%	-
2 and 4	GQ120042	Cnidaria	Hydrozoa	*Rosacea cymbiformis*	NGS/cloning	≥98%	-
3 and 4	JQ306039	Arthropoda	Malacostraca	*Polybius henslowii*	NGS/cloning	≥98%	-
3 and 4	KF483708.1	Arthropoda	Maxillopoda	*Caligus sclerotinosus*	NGS	≥70%	≥95%
1	HM593055	Chordata	Actinopterygii	*Scomber japonicus*	NGS	≥98%	-
1	KC015306	Chordata	Actinopterygii	*Cubiceps gracilis*	NGS	≥98%	-
1	KC860970	Mollusca	Cephalopoda	*Mastigoteuthis atlantica*	NGS	≥98%	-
1	AY047604	Arthropoda	Malacostraca	*Thysanoessa gregaria*	NGS	≥98%	-
1	FJ581756	Arthropoda	Malacostraca	*Meganyctiphanes norvegica*	NGS	≥98%	-
1	EF989676.1	Arthropoda	Malacostraca	*Cystisoma pellucida*	NGS	≥70%	≥95%
2	KC409639	Chordata	Actinopterygii	*Sparus aurata*	NGS	≥98%	-

2	KJ709658	Chordata	Actinopterygii	*Trachurus picturatus*	NGS	≥98%	-
2	HM007765	Chordata	Actinopterygii	*Lophius (genus)*	NGS	≥98%	-
2	DQ864410	Mollusca	Bivalvia	*Mytilus galloprovincialis*	NGS	≥98%	-
2	KC774030	Mollusca	Gastropoda	*Cavolinia inflexa*	NGS	≥98%	-
2	JQ306144	Arthropoda	Malacostraca	*Jaxea nocturna*	NGS	≥98%	-
2	JQ305932	Arthropoda	Malacostraca	*Plesionika narval*	NGS	≥98%	-
2	JN591694	Arthropoda	Malacostraca	*Goneplax rhomboides*	NGS	≥98%	-
2	FJ876945.1	Mollusca	Gastropoda	*Pneumoderma violaceum*	NGS	≥70%	≥95%
2	AY386273.1	Arthropoda	Maxillopoda	*Caligus elongatus*	NGS	≥70%	≥95%
2	GQ119963.1	Cnidaria	Hydrozoa	*Ceratocymba (genus)*	NGS	≥70%	≥95%
2	*	Arthropoda	Malacostraca	*Euphausiidae (family)*	NGS	≥70%	≥95%
2	*	Arthropoda	Malacostraca	*Polybiidae (family)*	NGS	≥70%	≥95%
3	EU148247	Chordata	Actinopterygii	*Maurolicus muelleri*	NGS	≥98%	-
3	AB488406	Chordata	Actinopterygii	*Scomber colias*	cloning	≥98%	-
3	KC287552	Arthropoda	Maxillopoda	*Clausocalanus furcatus*	NGS	≥98%	-
3	*	Arthropoda	Malacostraca	*Nyctiphanes (genus)*	NGS	≥70%	≥95%
3	AY937370	Cnidaria	Hydrozoa	*Forskalia tholoides*	NGS	≥70%	≥95%
3	KC545792	Arthropoda	Malacostraca	*Solenocera crassicornis*	NGS	≥70%	≥95%
4	*	Cnidaria	Scyphozoa	-	NGS	≥70%	≥95%
4	GQ120050	Cnidaria	Hydrozoa	*Sulculeolaria quadrivalvis*	cloning	≥98%	-

**No accession number is given if probability of belonging to a certain species is lower than 95% threshold.*

4.4.5 Prey composition with respect to sunfish size class

Overall, ocean sunfish stomach contents sampled in this study were dominated by Malacostraca, Actinopterygii and Hydrozoan prey. Interestingly, we found different diet preferences related to size (Figure 22). Molecular data shows that sunfish larger than 0.80 m have a more restricted diet, a trend that was also evident during stomach dissection as no hard parts (e.g. carapace, appendages) from prey were present in larger fish. The number of sampled fish captured belonging to size class 4 was however smaller than for the remaining classes. The highest prey diversity was found in fish belonging to size class 2 (0.40 – 0.60 m), which was also the size class with more stomachs sampled (and was the predominant size class found within the set-net). Specifically, small sunfish individuals (Class 1 < 0.40 m) were found to consume prey belonging to five different taxonomic classes: Malacostraca (40%); Actinopterygii (33%); Maxillopoda (13%), Hydrozoa and Cephalopoda (7% each), whereas size class 2 individuals consumed prey from six different taxonomic classes: Malacostraca (40%), Actinopterygii (20%), Hydrozoans (15%) and Gastropoda (10%), Maxillopoda (10%) and Bivalvia (6%). Sunfish of size class 3 (0.60 – 0.80 m) consumed prey from four different taxonomic classes: Actinopterygii (29 %), Malacostraca (29%); Maxillopoda (24%) and Hydrozoans (18%). In contrast, larger sunfishes (TL > 0.80 m) showed a more restricted diet that consisted of Hydrozoa (43%), Malacostraca (29%), Scyphozoa (14%) and Maxillopoda (14%) (Figure 22).

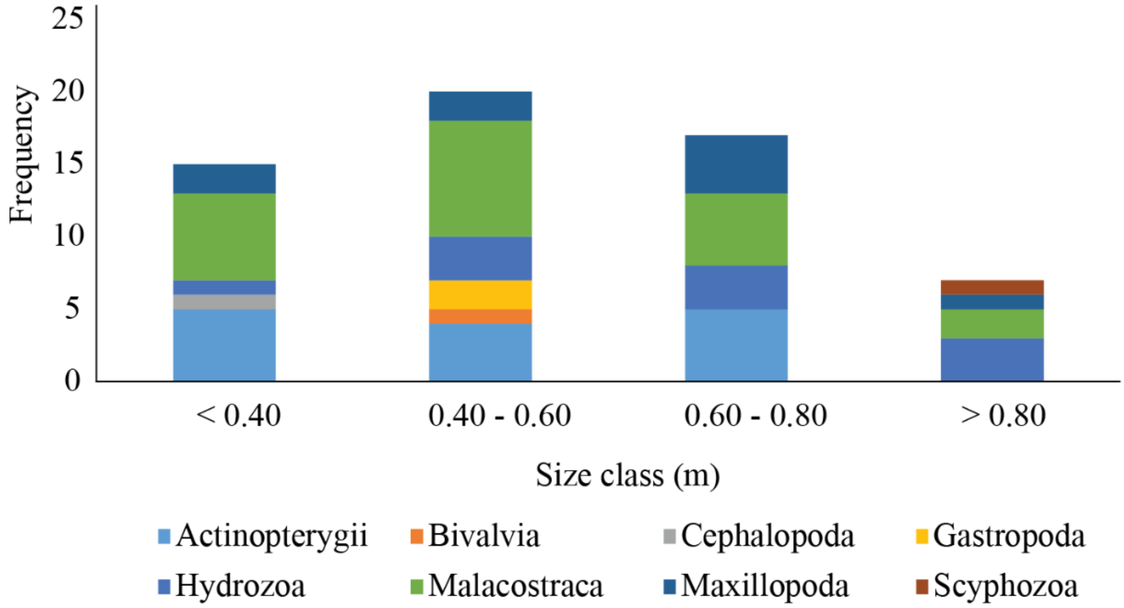

Figure 22 Barplot showing the different taxonomic groups consumed per sunfish size class (in metres).

The pelagic amphipoda *Phrosina semilunata*, the siphonophore *Physophora hydrostatica*, the deep water fish *Hygophum benoiti* and the sea lice *Lepeophtheirus pollachiu* were found in fish from three out of the four size classes, and are probably prevalent in the sunfish diet. Some other species which were found to be common to at least two size classes were the crustaceans *Funchalia villosa, Pasiphaea sivado, Polybius henslowii, Liocarcinus holsatus, Caligus sclerotinosus* and another unidentified *Caligus sp;* the teleost fish *Conger conger* and another unidentified teleost; and the hydrozoans *Rosacea cymbiformis* and another unidentified species.

Finally, results obtained using Bray–Curtis dissimilarity, which ranges from 0 (no difference) to 1 (total difference), indicate that although the diet of smaller sized fish (below 0.80 m) was more diverse (Table 16, dietary dissimilarities were lower (i.e., diets were more similar) between these size classes (for classes 1, 2 and 3: mean dissimilarity = 0.30) and higher between these smaller size classes and the larger individuals (on average 0.54 dissimilar, Table 16).

Table 16 Bray - Curtis dissimilarity matrix (weighted means) for diet composition among different sunfish size classes (low values = high overlap).

	< 0.40 m	**0.40 – 0.60 m**	**0.60-0.80 m**
0.40 – 0.60 m	0.31		
0.60-0.80 m	0.28	0.29	
> 0.80 m	0.56	0.58	0.48

4.5 Discussion

Sunfish dietary habits was determined in this study, using a molecular barcoding approach to identify consumed prey from stomach contents. From the 100 juvenile and subadult sunfish sampled, DNA from the stomach contents of 57 individuals was successfully amplified. We were able to assign barcoding sequences to a species or genus for 63% of the retrieved data. This level of sequence recovery was similar to the levels of prey recovered using metabarcoding of the faeces of an omnivorous terrestrial mammal, the brown bear, using several group specific primer markers (De Barba, Miquel et al. 2014). In total, 41 different prey items were identified belonging to 5 different phyla, which confirms that overall the sunfish is a generalist predator. Our data suggest an ontogenetic shift in dietary preferences: juveniles consumed teleost fish, Hydrozoa, Malacostraca, Maxillopoda, Bivalvia, Gastropoda and Cephalopoda prey, whereas subadults seem to be more selective, feeding mainly on Malacostraca, Hydrozoan and Scyphozoa. Taken together, our results contradict the classical view of sunfish being an obligatory gelatinous plankton feeder, supporting the findings of more recent studies (Pope, Hays et al. 2010, Syvaranta, Harrod et al. 2012, Harrod, Syvaranta et al., 2013, Nakamura and Sato 2014).

The scarcity of larger gelatinous prey in our results is, however, somewhat unexpected since these have been suggested to be predated by sunfish (Pope, Hays et al. 2010, Nakamura, Goto et al. 2015). One explanation for this apparent lack of large gelatinous species in the diets of sunfish may be due to the high digestibility of these soft-bodied animals and consequent rapid DNA degradation (Bowser, Diamond et al. 2013). In fact, amplification of the siphonophore

Sulculeolaria quadrivalvis occurred only at an earlier stage of this work, which suggests that DNA from these gelatinous prey was in fact degraded. The fact that no adults (> 1.40m Nakatsubo, Kawacxt et al. 2007) were sampled could also help to explain these results, as predation on large syphozoa was only observed in previous studies for larger individuals (Nakamura, Goto et al. 2015).

4.5.1 Ontogenetic differences in diet composition probably reflect habitat occupancy

One of the goals in studying the diet of marine species, besides ascertaining its trophic position and predator-prey interactions, is to investigate habitat use, as critical foraging habitats may be revealed through insights into dietary habits of species (Peters, Ophelkeller et al. 2014). The diversity of prey taxa present in sunfish stomach contents indicates that the species explores both benthic and pelagic food resources in the north-east Atlantic. Even though no adult fish were sampled in this study, we found evidence for an ontogenetic diet shift from juveniles to subadults, which confirms the results advanced by SIA analysis performed elsewhere (Syvaranta, Harrod et al. 2012, Nakamura and Sato 2014). Interestingly, Malacostraca and Maxillopoda crustaceans were found in stomachs of sunfish of all size classes included in the analysis, comprising between 29 and 40% and between 10 and 24%, respectively, of the sampled diet. While Actinopterygii appear to be a key component of the diet of juveniles (representing between 20 and 30% of the total items found), they were absent from the stomachs of subadults. Similarly, Cephalopoda, Bivalvia and Gastropoda only appeared to be consumed by juvenile sunfish of smaller sizes (Classes 1 and 2: 0.40 – 0.60 m), while cnidarians seem to be key in subadult diets (Class 4: >0.80m), totalling about 57% of consumed prey. Furthermore, the Bray – Curtis dissimilarity matrix revealed an increased similarity between the diet composition of juveniles when compared to subadults. Ontogenetic shifts in the diet of teleost species appears to be a common trait, and have been previously found for the yellowfin tuna (*Thunnus albacares*) (Graham, Grubbs et al. 2007), hake (*Merluccius merluccius*) (Mahe, Amara et al.

2007) and the red mullet (*Mullus surmuletus)* (Labropoulou, Machias et al. 1997). In the case of the sunfish, smaller fish seem to have a broader trophic niche than larger fish, probably feeding opportunistically on what is available in the habitats they occupy.

The presence of coastal prey in smaller sunfish, such as the euphausiacea *Thysanoessa gregariai,* the burrowing shrimp *Jaxea nocturna*, the shelf/slope waters northern krill *Meganyctiphanes norvegica*, the benthic crab *Goneplax rhomboids,* and *Mytilus galloprovincialis* suggests a juvenile preference for coastal habitats (Nickell and Atkinson 1995, Brinton, Ohman et al. 2000, Cuzin-Roudy 2010). In addition, all the teleost species found to be consumed by juveniles are known to spawn along the coasts of the Gulf of Cadiz and western Iberia in a time frame that is coincident with our sampling, or remain in shallow coastal waters for long periods of time [e.g *H. benoitti* (Olivar and Palomera 1994); the *Trachurus picturatus* (Abaunza, Murta et al. 2008); the *Sparus aurata* (Sheaves 2006) and *Conger conger* (Correia, Isidro et al. 2002)]. Recent video recordings of small sunfish show that they feed on benthic coastal crustaceans (Nakamura and Sato 2014). In the present study however, some pelagic prey items were also recovered from the stomach of smaller sunfish such as the mesopelagic teleost *Maurolicus muelleri,* the leptocephalus eel larvae, the decapod *Pasiphaea sivado,* and the crab *P. henslowii* (Rodriguez, Gonzalez-Nuevo et al. 2009, Cartes, Papiol et al. 2014), indicating that they might also be actively feeding in the water column. In the case of sunfish of larger sizes (class 4), all prey species identified were pelagic: the decapod crab *P. henslowii*, the siphonophore *Physophora hydrostatica,* the amphipod *Phrosina semilunata,* the epiplanktonic nectophore *Rosacea cymbiformis* (Bouillon, Medel et al. 2004) and the siphonophore *Sulculeolaria quadrivalvis* (Licandro, Souissi et al. 2012). Moreover, the single scyphozoan detected from sunfish stomach contents was found in individuals of sizes larger than 0.80m. Hence, the exclusive incidence of pelagic species in these larger fish seems to confirm their dependency on the water column to feed (Nakamura, Goto et al. 2015).

The data presented here, together with the prevalence of small sized sunfish in the set-net, is consistent with the results from a recent satellite tracking study for this species in the north-east Atlantic, where we described a more coastal occurrence of smaller sized fish when compared to larger individuals (Chapter 3). Moreover, the same study described the occurrence of both normal and reverse diel vertical migration (DVM) together with surface oriented and an irregular behavioural pattern in the depth occupancy of sunfish. In that study, depth was recorded for sunfish of sizes not exceeding 1.00 m and recorded movements seem to conform to the behaviour of opportunistic feeding. The study of Nakamura *et al.* (2015) focusing on larger sunfish (>1.4 m), showed that these respond to patchily distributed prey in the water column, mimicking the DVM behaviour found for many gelatinous predators (Hays, Hobson et al. 2006, Houghton, Doyle et al. 2008). Taken together, these alternate vertical movements may well be a reflection of the foraging habits of a generalist predator.

4.5.2 NGS vs. Cloning

Our results support the superior performance of NGS compared with cloning techniques. Besides the technical difficulties inherent to cloning (e.g. transforming competent cells), accurate diet characterization depends on the number of clones selected for sequencing. Briefly, in the present study, when using a DNA cloning and sequencing approach, eight prey species were identified from a total of 33 samples (Table 15). Comparatively, even before employing Bayesian statistics to identify a larger number of items, 25 prey species were identified from 36 individual stomach contents via NGS metabarcoding (Table 15).

Nevertheless, some studies have been successful in assessing diet of marine organisms using cloning, for example in the case of the Steller sea lions (*Eumetopias jubatus* (Deagle, Tollit et al. 2005), where cloning was used to test the efficiency of DNA-based methods in diet reconstructions, and for the Australian sea lion, where 28 prey items were identified using cloning (Peters, Ophelkeller et al. 2014). Furthermore, the use of cloning even provided enough resolution to detect ontogenetic shifts in diet in the case of the largemouth bass (*Micropterus salmoides*), from which 26 prey species were retrieved (Jo, Gim et al. 2014). In the present study, cloning allowed the recognition of two prey species, the fish *Scomber colias* and the hydrozoan *Sulculeolaria quadrivalvis*, which were not detected by NGS. However, this could be due to the degradation of DNA following the frosting and defrosting after the first PCR reaction (for cloning). Another explanation is that different PCR reactions amplified DNA from different prey species due to the known possible bias of primers at binding sites, which could make some prey species more prone to amplification than others (e.g. Boyer, Wratten et al. 2013). In our study for the same sample, we included as many repetitions as possible of PCR products which were then purified and quantified before sent for NGS.

4.5.3 Technical caveats: from marker choice to the use of universal and the necessity of blocking primers

Since 2003, when the cytochrome oxidase subunit I (COI) was proposed as a barcoding tool (Hebert, Cywinska et al. 2003), huge efforts have been made to collect and reunite DNA data from all living organisms in public databases. As a result, for example, the BOLD database comprises now more than 3.7 million COI sequences. For this reason, and despite some dispute regarding the applicability of COI for amplicon-based metabarcoding (e.g. Deagle, Jarman et al. 2014), it remains the best option for metabarcoding.

The usage of universal primers that bind to highly conserved regions across taxa is a premise in metabarcoding studies surpassing the use of group-specific primers in terms of cost and time-effectiveness (Blankenship and Yayanos 2005, Bowser, Diamond et al. 2013). However, their use in dietary studies is not without the problems that arise by concurrent amplification of the predator's DNA (Boyer, Cruickshank et al. 2015). For this reason, we opted to employ a blocking primer to prevent extension of the sunfish DNA during PCR amplification. This reduced to less than 5% the number of sequences obtained for the sunfish (vs. >60% prior to its usage), greatly improving the successful recovery of prey sequences. Similarly, Vestheim & Jarman (2008) were able to reduce predator amplicon to as low as 2% by adding blocking primer to PCR reaction. Hence, although our results highlight the usefulness of metabarcoding to determine diet composition of marine species, encouraging its use in future ecological studies, the use of predator-specific blocking primers during PCR amplification seems to be crucial to avoid host co-amplification, especially when using universal primers that target the barcoding region of the COI.

Chapter 5: Inferences on predator-prey interactions of the World’s largest teleost in the north-east Atlantic

5.1 Abstract

The understanding of important ecological marine interactions have been hampered by the lack of understanding on how marine predators perceive the environment. Yet, the space use by predators is expected to reflect both the distribution and availability of prey. This is the case for planktivorous species feeding on assemblages of prey that are patchily distributed, and tend to aggregate forming biological ‘hotspots’ in the open ocean. This study reports the estimation of a plankton density index for a defined geographic area in the north-east Atlantic using a model of ocean currents at very fine resolution (~2 km). Movements of tracked ocean sunfish were related to these model-generated potential prey ‘hotspots’ and consequently the relative foraging success that may take place was estimated. Interestingly, overall, sunfish spent more time in regions of model increased plankton

density. By comparing real sunfish paths with 100 null tracks per individual, the structure of tracked sunfish movements resulted in an average of 59% higher simulated plankton encounters. Importantly, a seasonal pattern was identified with sunfish performing no better than random in productive seasons, whereas in winter and autumn months, real tracked fish outperformed the random tracks. On the other hand, foraging patterns of sunfish typified by area-restricted searching (ARS) were identified in Fastloc-GPS^{TM} tracked sunfish movements, at spatial scales smaller than 7 km. Thus, these ARS behaviours were environmentally integrated with the estimated plankton density maps, sea surface temperature, thermal gradients and chlorophyll *a*. Sunfish monthly-scale ARS spatially overlaps with peaks in both generated plankton densities and thermal gradients; whereas daily distance to maximum plankton densities significantly decrease when exhibiting ARS in the north-east Atlantic. These patterns were further confirmed by a GAMM model which revealed that sunfish ARS occurs within a thermal envelope between 17 – 22ºC, sharp gradients in thermal features and increased aggregations of potential prey fields.

5.2 Background

Preferred habitats are often revealed by the movement patterns of a species, allowing the determination of critical areas occupied for breeding, feeding and for protection or shelter (Benhamou and Cornélis 2010, Simpfendorfer, Yeiser et al. 2011). In the marine environment, foraging usually implies the searching for and, when found, exploitation of patchily distributed resources. But how marine predators perceive the dynamic environment they occupy and respond to it remains poorly understood (Fauchald 1999). Therefore, a better understanding of both spatial and temporal utilisation of the environment by an organism will lead to a more complete description of the processes underpinning important ecological interactions. Marine predators are generally acknowledged to spend more time in regions of increased abundance of resources (Fauchald 1999, Fauchald and Tveraa 2003, Bestley, Patterson et al. 2010), being highly dependent on the distribution of prey. Furthermore, high consumption rates of dense prey fields, and consequent increased energy intake, will often drive the aggregation of predators in prey 'hotspots' (Hays, Hobson et al. 2006, Scales, Miller et al., 2014 and 2015). However, identification of feeding areas through direct observation of pelagic species is difficult in the marine environment.

The spatial accuracy of individual movement patterns using such technological approaches has often been too coarse for fine scale movements performed by the animals to be recorded. The advent of Fastloc-GPS^{TM} tags has enabled the high-resolution recording of marine species space-use, due to the increased accuracy of locations retrieved (Hazel 2009, Kuhn, Johnson et al. 2009, Sims, Queiroz et al. 2009, Cagnacci, Boitani et al. 2010, Frair, Fieberg et al. 2010, Hebblewhite and Haydon 2010, Kuhn, Tremblay et al. 2010, Merrill, Sand et al. 2010). Owing to this advanced tracking system, it is now possible to identify fine scale behaviours of large vertebrate species that surface briefly reliably, such as area-restricted searching movements that are characteristic of foraging. Critically, the mechanistic processes underpinning a species' decision to stay or leave an area remain little known for most marine species (Sims 2010). However, it is expected that when within a profitable prey patch, predators remain to exploit the resources, presumably maintaining consumption rates above a lower threshold until this threshold is reached, either through direct depletion or through the interference competition from conspecifics or other species (e.g. Hays, Hobson et al. 2006).

Plankton comprises the foundation of several marine trophic food webs. Examples of a planktivorous feeding strategy include large top predators such as baleen whales (Richardson 2008), sea turtles (Hays, Hobson et al. 2006) and several species of shark and ray (Clark and Nelson 1997, Sims 1999, Sims, Witt et al. 2006). All these species, which are among the largest marine organisms, rely on consumption of large amounts of zooplankton that provide not only energy for increased growth and ultimately reproduction, but also sufficient for large-scale movements or migrations. When a prey field is highly changeable such as with zooplanktonic patches, marine predator behavioural strategies should rapidly change in response to different food concentrations (Bartumeus, Peters et al. 2003). For example, tracked planktivorous basking sharks (*Cetorhinus maximus*) moving within zooplankton prey fields ceased filter feeding when densities fell below the predicted lower threshold (<0.6 mg m^3) (Sims 1999). Indeed, it is this threshold, of when to leave a patch before feeding conditions deteriorate, that will help determine the survival of organisms. Although not exclusively planktivorous, the ocean sunfish (*Mola mola*) is also dependent on assemblages of zooplanktonic species (e.g. Dewar, Thys et al. 2010, Pope, Hays et al. 2010, Nakamura and Sato 2014). Therefore, exploring the linkage between sunfish and plankton distribution is important within the context of marine predator-prey interactions. Several studies have quantitatively linked the observed animal paths and behaviours to biological or physical oceanographic features (Sims 1999, Pinaud and Weimerskirch 2007, Weimerskirch 2007, Freitas, Kovacs et al. 2008, Miller & Priede 2009), even though the number of studies focused on fine-scale patterns often include large-scale oceanographic features (Hamer, Humphreys et al. 2009).

The most complete geographic plankton database in the North Atlantic is the continuous plankton recorder (CPR) survey (Richardson, Walne et al. 2006). This plankton sampler is opportunistically towed at ~7m depth behind commercial ships operating in the area and has recorded the abundance of near-surface zooplankton in the north-east Atlantic and North Sea since 1931. The resulting data have been extensively used to study plankton biodiversity and climate change effects (e.g. Beaugrand, Ibañez et al. 2003 and studies referenced within, Richardson, Walne et al. 2006). Different studies have employed known plankton distributional datasets, as the CPR, and linked the spatial coherence between predator movements and prey-fields, obtained for the same geographic area (Sims, Witt et al. 2006). However, tracked sunfish movements (Chapters 2 and 3) do not overlap with CPR sampling routes which precludes direct comparison of predators and prey fields. Consequently, this study made use of a very fine-scale model of geostrophic currents to simulate the aggregation of planktonic species for the same region occupied by tracked sunfish, with a view to testing hypotheses about sunfish foraging habitat selection.

Several oceanographic features are known to influence the distribution of organisms in the marine ecosystem. With regards to the patchiness of plankton, different explanatory hypotheses have been advanced (see Brentnall, Richards et al. 2003 for a review). The transport of a patch to another previously unoccupied location (*advection*) should be interpreted separately from *concentration* (the change of a patch's density), with both having different physical or biological drivers (Graham, Pagès et al. 2001). Within the scope of this study advection *per se* is the focus, where currents force the dispersal of plankton masses. Importantly, zooplankton and larvae are likely to be moved by ocean currents as these physical processes are known to drive the distribution of organisms with limited swimming capabilities (McManus and Woodson 2012). In fact, free-floating plankton is expected to show strong distributional patterns with oceanic currents (Richardson 2008). Importantly, mesoscale anticyclonic eddies, considered to be critical oceanic biological 'hotspots' (Bakun 2006, Doyle, Houghton et al. 2008) were found to significantly dictate the distribution of plankton species within the Gulf of Alaska for instance (Batten and Crawford 2005). Advection was found to be an important explanatory factor for the interannual variability in the abundance of gelatinous zooplankton in the northern North Sea (Lynam, Attrill et al. 2009), although recent individual tracking of large jellyfish indicates their ability to resist such physically mediated dispersal by active counter-current swimming (Fossette, Gleiss et al. 2015). Generally, foraging of marine predators tends to occur in highly dynamic regions including fast currents (e.g. Gaspar, Georges et al. 2006, Fossette, Girard et al. 2010), supporting the general observation that ocean geostrophic features modulate zooplankton distribution by aggregating it in specific zones which, consequently, dictates the structure of patchiness in oceanic prey fields.

In this study, the movement patterns of tagged sunfish (*n* = 18) were investigated in relation to an estimated distribution of planktonic species in the region. A model of currents at fine resolution (~2 km) was used to explore physically mediated biological 'hotspots' of potential prey of sunfish. In particular, the plankton index encountered by the fish (estimated foraging success) during the tracked displacements in comparison to random movements at similar temporal and spatial scales, was computed. Thus, we aim to test the hypothesis that broad-scale movements of sunfish target areas of enhanced plankton densities. Furthermore, ARS behaviour along GPS-tracked sunfish trajectories (*n* = 7) was integrated with the simulated patchy environment, with the aim to explore potential clues (relating to where) and cues (relating to when) that this pelagic predator might be responding to at the very fine scale. Different studies have revealed large-scale worldwide movements of this species (Cartamil and Lowe 2004, Sims, Queiroz et al. 2009, Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010, Potter, Galuardi et al. 2010), but none have focused on the fine-scale foraging patterns.

5.3 Methods

5.3.1 Plankton density (PD) simulation

The high-resolution (eddy-resolving) NEMO model at 1/12º (2007-2011) and 1/36º (2012-onwards) horizontal resolution with hourly time-steps comprises physical variables provided by the *MyOcean IBI-MFC for the Atlantic-Iberian Biscay Irish- Ocean*. This product of hourly means of surface fields (sea surface height (SSH), temperature (SST) and currents, together with barotropic velocities) informed the plankton density simulated maps. Hence, using hourly information of both *u* and *v* velocity components we simulated the ratio of particles (start=1000) displacement per grid cell (PD index), using a custom written software *(MBA Plankton densities simulator).* Simulations were performed for the tracking period [2007-2013] at monthly intervals, resetting the starting random distribution of particles within the grid at each month. Lastly, monthly maps of likely free-floating prey aggregation areas were then computed and the respective PD index was extracted for every real and random sunfish position.

5.3.2 Plankton density validation

Prior to further analysis, the simulated PD index was validated using information gathered by the CPR dataset on presence/absence of gelatinous species (Cnidaria, Siphonophorae and Thaliacea presence). Here, both CPR information on gelatinous species total occurrence for the period between 2007 and 2013 were gridded at 25 km grid cells and overlaid with the maps generated for the PD index, with similar spatial resolution. Maximum values of both CPR gelatinous occurrence and PD index for the same spatial area were computed and correlations between these two were then calculated. Further comparisons of PD index with values of SSH at the very fine scale of 2 km were also performed. We expected a good correspondence between PD and eddies for sufficient validation of the PD simulator.

5.3.3 Sunfish residency in relation to the simulated PD

Linear modelling of sunfish residency estimated by counting all daily tracked sunfish positions per 1º grid cell was performed. To standardise, resultant summed time spent was weighed by number of simultaneous fish on respective dates, so two tags count = 0.5 each and so on (1/no. simultaneous tags). *Sum count* was the dependent variable and results from the sum of sunfish weighted positions within that cell, whereas *Max plankton* was the independent factor (predictor variable) of the maximum plankton density (PD) for that cell.

5.3.4 Real and null sunfish tracks

Foraging success (measured as theoretical ‘prey’ encounters) of tracked sunfish within a computer generated model prey field was assessed by comparing real track performances with that of model random fish trajectories. Briefly, satellite-retrieved sunfish tracks were reconstructed and interpolated into daily paths using a Kalman filter as described previously (Chapter 3). To avoid inaccurate interpolations, gaps exceeding 20 days were removed and respective tracks were split into sections beforehand.

Subsequently, a total of 100 positions were generated per real sunfish daily location within a 1º radii (cell) using a uniform distribution. This step allowed the generation of 100 random tracks according to the same statistical parameters as the real sunfish movements. Thus, the available environment that was not actively utilised by the sunfish was assessed with model tracks and use to test the performance of the species’ foraging success.

Sunfish residency was calculated as a function of the simulated PD index. Here real tracks were gridded, weighted by the number of simultaneous fish in each cell on that respective date (as described above), for a 1º grid cell size (which encompasses all tracking errors). Thus, centroid summed locations were counted and statistics (mean and maximum) of simulated PD index were assigned to each grid cell. A linear model was applied to the time spent by tracked sunfish (number of daily positions) in relation to maximum plankton densities, after excluding all possible correlation structure in the data.

5.3.5 Real vs. random encountered PD

To investigate sunfish foraging success in relation to the simulated PD index, we tested the modelled densities encountered along the actual tracks (averaged for each tracking system accuracy) against the index available for that position. To account for tracking system spatial inaccuracy, we resampled all real fish daily locations (*n* = 100 per real position) from within the mean error radius of each inherent tracking error: PSAT (1.02º latitude and 0.54º longitude, Sippel, Holdsworth et al. 2011); or Argos (0.12º Patterson, McConnell et al. 2010). Average environmental extraction of all generated positions was then assigned to each sunfish position. For the random null tracks, modelled PD was extracted for each of the random locations uniformly generated within each 1º cell radii. Final comparisons were performed to determine overall success and also at both seasonal and monthly temporal scales.

Also, assuming that particles are being consumed, total amounts of PD per track (real and null) were calculated. Therefore, absolute theoretical plankton encountered by our tracked fish were used to estimate the individual and overall differences in foraging success within the visited regions; these were then compared to the same metrics for the random simulated tracks generated.

5.3.6 Sunfish Area-Restricted Searching (ARS)

A total of eight sunfish were simultaneously tracked via Argos and GPS, the latter data providing locations with high accuracy (< 60 m) and thus were analysed for possible ARS patterns. All tracks were linearly interpolated and were analysed in a per point time inspection. Intra-individual variation in the time between consecutive positions were found using a Kruskal-Wallis test (H = 67.236, df = 7, $p < 0.001$). A multiple comparison procedure (Dunn's method), revealed two individuals to have different gap intervals at the 0.05 level of significance (#14 and #6). Sunfish #14 track was thus removed from the analysis whereas #6 was maintained since the average gap was smaller in relation to all the remaining six individuals, and thus introduced no larger error into the analysis. Therefore, considering only the six non-significantly different tracks, in terms of time between positions, the average time step was 0.45 days ± 1.71 (mean ± s.d). To ensure at least one retrieved position was always incorporated, overall trajectories were interpolated into regular tracks at every 12 hours. Lastly, to avoid the introduction of additional errors inherent to the interpolation, GPS positions more than 3 days apart were not interpolated and track sections with less than three positions were discarded from the analysis.

For every individual sunfish track, the speed (or speed over the ground) was computed for segments with gaps not exceeding 3 days. To normalise between tracks, the first quartile of speeds (25%) was used as a threshold (Weng, Foley et al. 2008). Individual track shape (degree of tortuosity) was investigated using first passage time (FPT), using a custom written software package (*Track Analysis v.4, Marine Biological Association, 2009*). Briefly, FPT was calculated at each interpolated location along the track for radii ranging from 1000 m to a maximum corresponding to half of the track length, incrementing at 1000 m. Log-transformed variance of FPT was plotted as a function of the radius size (ensuring the independence of the magnitude of the mean first-passage time, Fauchald and Tveraa 2003) and ARS scale was identified for each individual track as the maximum peak in this log-transformed variance, the known scale at which sunfish increased its search effort. Lastly, a straightness index was calculated for all sunfish tracks to detect track segments with higher turning frequency and where such movement occurs temporally with the track. This index was calculated as the ratio between the straight line displacements over a week and the travelled distance within the same period, in km, in such a way that the closer to 1 the computed value, the straighter the movement was. Weekly periods were selected to match weekly averages of oceanographic data used.

Discrete information on speed, sinuosity index and FPT was used to inform whether ARS behaviour occurred along the track, with ARS being a proxy for foraging movements leading to prey consumption. All three parameters were closely connected with higher tortuosity expected to occur when the animal turned more frequently (possibly also swimming at lower speed), which increased the time needed to cross a specified area. However, no colinearity was found between the three indexes with all coefficients in the Spearman's rank correlation matrix not exceeding 0.75 (Zydelis, Lewison et al. 2011). In summary, combined pairs of at least two positions within the first quartile for speed and/or sinuosity (low velocities and higher tortuosity) and the third for FPT, were assigned as ARS. For this study, ARS was accepted if one of the three parameters fell in between the thresholds, and detected intense search in contrast to the faster and more linear trajectory inherent in travelling/commuting animals.

5.3.7 Fine-scale environmental integration of sunfish ARS behaviour

To investigate the potential environmental drivers of sunfish ARS we extracted fine-scale environmental features at every fish location, including both travel and ARS modes. Briefly, we used the eight day composite chlorophyll *a* concentration (Chl *a*) global product from L3 Ocean GlobColour EURO dataset[6], at both 4 km [2008-2009] and 2 km [2010 onwards] resolutions. Regarding the sea surface temperature (SST) , the eight days composite L3 MODIS AQUA product at 4 km for the period 2008 – 2011 was downloaded[7]; and the daily SST estimates from the NEMO model for oceanic

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[6] http://hermes.acri.fr/

[7] http://opendap.jpl.nasa.gov/opendap/

currents (used in the plankton densities estimation) at 2 km resolution was selected for the period 2012 – onwards. SST maximum gradients were calculated following methods described in Chapter 3.

Sunfish ARS was linked to the immediate PD over two spatio-temporal scales: (i) visually estimating a qualitative measure of the overlap between monthly maximum environmental features and sunfish behaviour (transit vs. ARS); and (ii) measuring the distance (in km) between sunfish and the daily maxima (within the attainable 20 km daily displacement by the fish (Sims, Queiroz et al. 2009)). In summary, monthly maxima of plankton densities for sunfish tracking period were estimated and overlap between sunfish ARS and this maxima relative index was individually performed. Furthermore, distances between GPS locations and a daily maximum of PD were calculated, within the estimated 20 km daily displacement, in relation to either ARS or travel modes.

Lastly, a generalised additive mixed model (GAMM) with binomial error and a logit link was used to estimate the probability of sunfish to perform ARS in relation to immediate SST, SST gradients, PD estimation and chl *a.* Models were fitted using the *gamm* function in the R package mgcv (Wood 2006) to include an autoregressive variance − covariance matrix (representing first-order continuous autocorrelation structure corCAR1 in R (R Development Core Team 2009), for modelling the serial dependency among observations for each individual (Zuur, Ieno et al. 2009). Colinearity among selected variables was analysed prior to model runs; and the dataset was split in 75% for training and the remaining 25% was used to validate the model by means of receiver operating curve (ROC), which illustrates the performance of the binary model. Briefly, the GAMM model structure was defined by: Behaviour ~ PD+ SST + SST_Slope + Chl*a*. The final model was selected by removing non-significant variables with confirmation of final model structure made with Akaike information criteria (AIC) based on weighted AIC using *akaike.weights* function in R package qpcR, (following Burnham and Anderson 2002). Model performance was attained by assessing the strength of correlation (*Pearson*) between the observed and predicted data together with the Hosmer and Lemeshow goodness of fit (GOF) test using the ResourceSelection package in R (Lele 2009).

5.4 Results

5.4.1 Validation of plankton simulations

Prior to relating sunfish movements to the monthly PD simulated maps (Figure S4), occurrence data on gelata from the CPR dataset (Cnidaria, Siphonophorae and Thaliacea presence) was used to validate the simulated output (Figure 23). We found prey totals correlated with maximum model-generated PD indexes (r_p= 0.400, p <0.001, n= 453) for the geographic region for which both CPR and simulated PD indices were simultaneously available. In addition, a positive correlation for the area was also found between maximum absolute values of SSH and $\log_{10}$ PD maximum (r_s= 0.249 and p <0.001, n= 541242) (Figure S5). This suggested that plankton, as simulated in this study, was potentially entrapped at both very high (+) or very low (-) SSH, which is aligned with the expectation that cyclonic and anticyclonic eddies tend to aggregate free-floating species. This result was further supported by a marginal but positive correlation between averaged plankton densities and absolute values of AVISO SSH at 25 km resolution (r_p = 0.033, p <0.05, n = 6448). Hence, our simulated prey field possessed features that accounted accurately for the oceanographic aggregation areas expected to concentrate buoyant organisms.

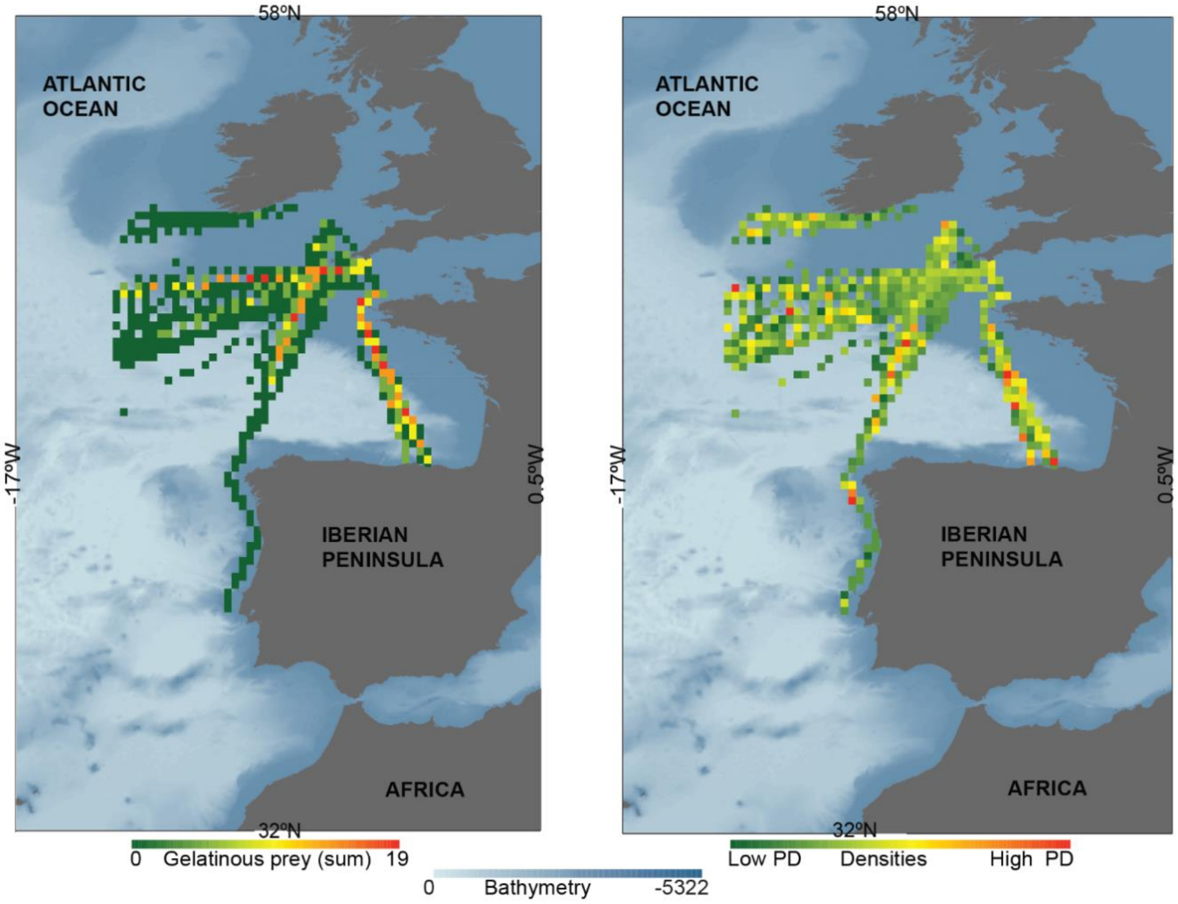

Figure 23 Maps representing gridded (25km) occupancy of both A) CPR summed gelatinous prey and B) maxima PD index estimated in this study, pooled from the concomitant period (2007-2011).

5.4.2 Sunfish residency in relation to the simulated PD

Overall, tracked sunfish were found to spend more time (significantly increased counts in a cell) in regions of elevated simulated PD (Adjusted R^2: 0.5395, $F= 71.3$, $p < 0.001$, $n= 63$). Model residuals (simple linear model as no autocorrelation was found in the sunfish positional dataset; Figure S6) ranged from -14.09 to 24.89 with a median of -0.55 and interquartile range of -2.95 and 2.44.

5.4.3 Real vs. random encountered PD

Significant differences were found between simulated PD encounters by sunfish tracked locations (Real PD) and null positions (Rand PD). Real fish movements overlapped more often with higher PD (median = 673 units) than at random (median = 575 units) (data non-normally distributed, Shapiro -Wilk $p < 0.050$; Mann-Whitney test for equivalence of medians: $W = 188875.5$, $p =$

0.0168). Taken together, not only did we find that sunfish residency overlapped with regions of likely higher prey aggregation but also, when compared to random, real tracks generally resulted in more higher density encounters. Figure 24 illustrates this increased encounter rate.

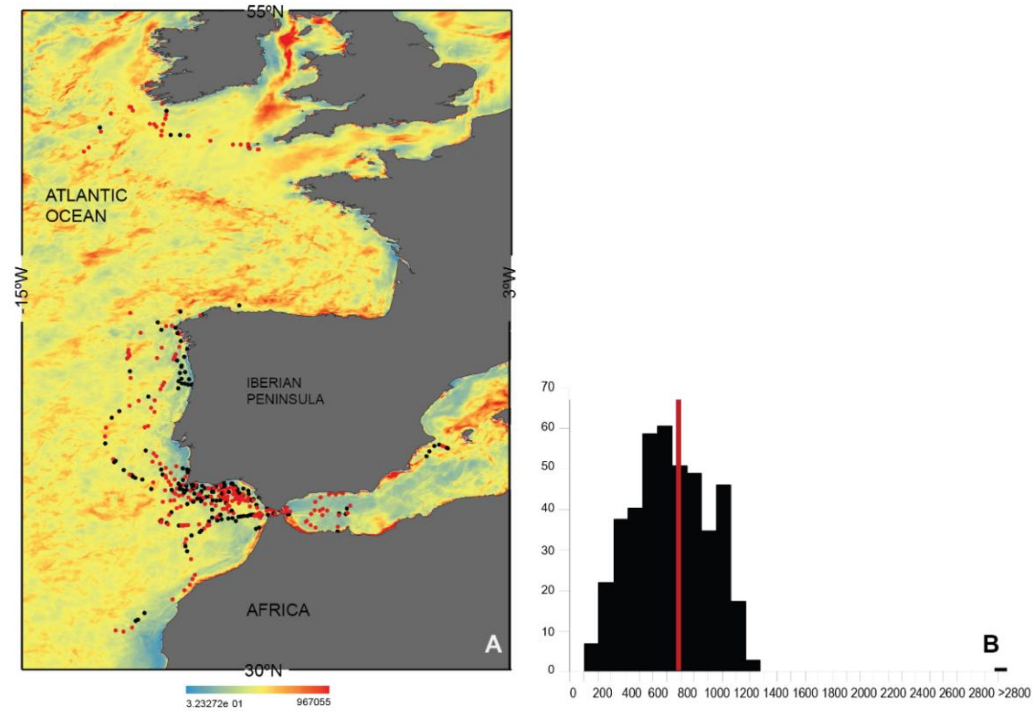

Figure 24 A) Map showing all tracks' positions coloured in red if real PD higher than the random and black otherwise, overlaid on the averaged plankton densities pooled from the entire tracking dataset. B) Histogram showing the average PD available (randomised locations – black) and the estimated to be encountered by real tracks - red bar.

In highly productive regions such as the west coast of the Iberian Peninsula during spring/summer months (due to intense upwelling), sunfish tracked closer inshore seemed to select lower than the average modelled PD. In contrast, offshore movements may have been tracking 'hotspots' that were correlated with the modelled PD. In the Cadiz Gulf, sunfish showed an increased PD simulated encounter, especially for fish that were actually dispersed from the tagging region, whereas 'resident' fish seemed not to track higher simulated PD in comparison to

Random PD. Importantly, tracked sunfish movements resulted in higher plankton encounters, on average, than 59% of the random simulated tracks.

5.4.3.1 Seasonality

An investigation into seasonal differences between real and random PD simulated encounters revealed sunfish movements exceed that available (averaged random tracks) in autumn/winter months, i.e. the colder months. In spring and summer, sunfish movements seem to be more strongly influenced by temperature (described in Chapter 3) and less by the plankton aggregation areas (Figure 25 and Figure S8 show seasonal patterns and maps of tracked individuals). Despite the different tracking years, results indicate a clear pattern of lower than average PD in summer months, whereas in colder periods sunfish locations coincided with increased simulated PD compared to that available in the model. This seasonal difference found in the simulation also reflects the increased productivity during summer months and the depletion in winter period (Figure S7).

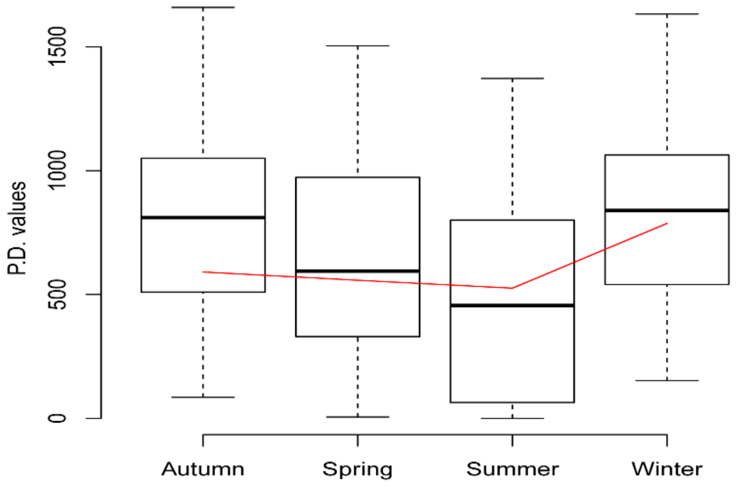

Figure 25 Boxplot of modelled seasonal PD index ‘encountered’ by tracked individuals with null median denoted by the red line.

Overall, we found that in relation to modelled PD, both summer and spring real sunfish movements performed no better than random (~54%), whereas in winter and autumn months, the simulated PD encountered by real sunfish tracks was higher than ~74% of the null tracks (autumn 69%; spring 57%; summer 50%; winter 78%). Analysis of variance revealed these ratios to be significantly different among seasons (normality test Shapiro – Wilk, *p* > 0.050; Kruskal-Wallis: H = 67.848, df = 3 and *p* <0.001).

5.4.3.2 Foraging success (consumption) estimation

Summed PD 'encountered' by tracked sunfish was greater on average than the mean encountered by the null tracks (15924.79 and 14748.07 units, respectively). Also, real sunfish had a higher 'consumption' of simulated plankton than 60% of the random tracks, suggesting that if the PD field is representative of real prey fields (as the CPR vs. PD correlation implies), then sunfish actual movements were structured in a way that led to more success in encountering aggregated particles (prey) (Figure 26). Individual sunfish tracks performance are given in Figure S9.

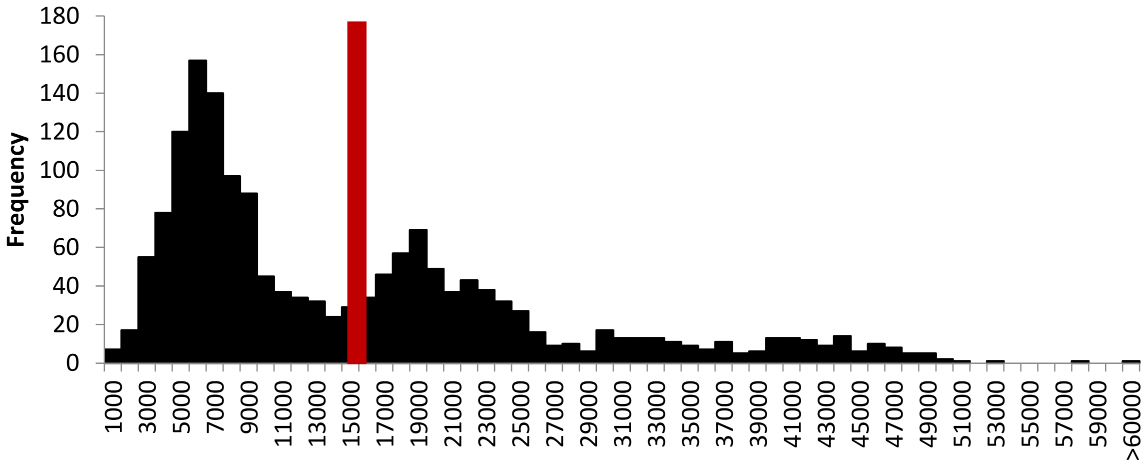

Figure 26 Histogram of random summed PD. Averaged real sunfish 'encountered' PD highlighted by the red bar.

Interestingly, although not significant (Mann-Whitney U = 84.0, *p* = 0.9296) real sunfish paths were found to encounter higher simulated PD, compared to null tracks, in lower available density environments (7406 units compared to 11235 - median). This result, albeit with few individuals, suggests that within areas of likely increased aggregation of prey, sunfish movements are no better than random trajectories; it is when prey is scarce that actual tracked trajectories show increased success rates of encountering simulated PD. We found no correlation between sunfish size and encountered PD compared to random (r_p= 0.081, *p* = 0.748, *n*= 18). Lastly, we can also exclude the track length as a potential confounding variable for this encounter success as no correlation was found between tracking times (days-at-liberty) and the ratio between real and random summed PD (r_p = 0.122, *p* = 0.629, *n*= 18).

5.4.4 Fine – scale behaviours of sunfish

With a maximum estimated track length of 1854 km, on average GPS tracked sunfish displaced 800 km from tagging region (

Table 17 and Figure 27). Overall, sunfish moved on average at speed of 0.40 m s^{-1} (± 0.49 ms^{-1} and a maximum of 7.61 ms^{-1}). Importantly, no GPS tag failed to transmit in this study.

Table 17 Individual sunfish GPS tracking information

S#	ID	Size (m)	No of Positions	Mean Gap (d)	Std. Error	Date Tagged	Tracked Period (d)	Track length (km)
5	75760	0.60	46	0.336	0.114	14/05/2008	15	134.6
6	75761	0.60	150	0.031	0.008	14/05/2008	5	85.0
8	75763	1.00	232	0.400	0.075	06/11/2008	92	1601.2
9	75762	1.00	206	0.795	0.209	18/05/2010	163	1853.6
10	99102	0.97	4	0.735	0.519	21/05/2010	10	98.0
14	15122	1.00	8	5.665	1.584	18/05/2012	51	432.2
17	133671	1.25	116	0.638	0.136	14/10/2013	73	835.7
18	133672	1.15	144	0.332	0.082	19/10/2013	48	1090.6

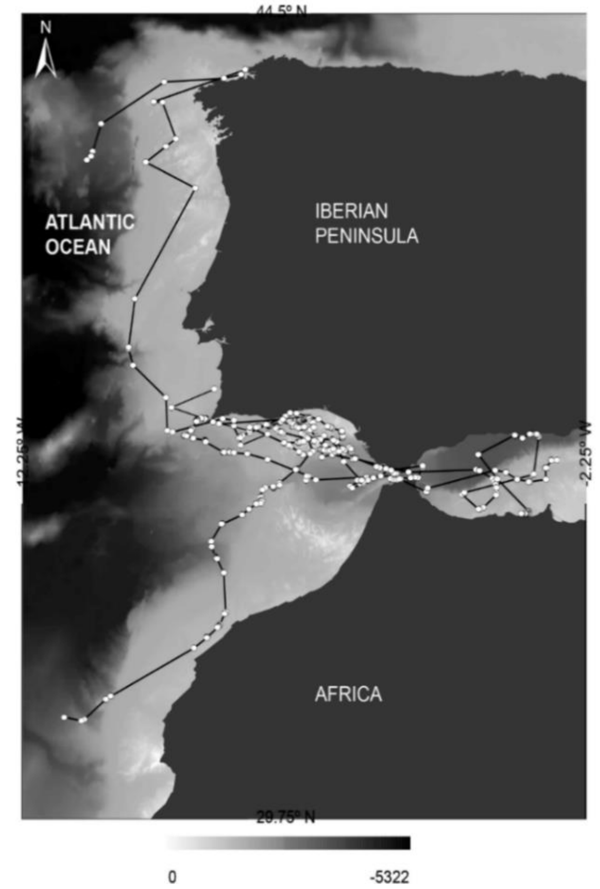

Figure 27 Map of all eight Fastloc-GPS^{TM} tracked sunfish processed locations (white dots) with black line denoting the linear interpolated track, overlaid in a bathymetric map.

5.4.4.1 Sunfish Area restricted search behaviour

There were no peaks in the variance of FPT in sunfish tracks #S6 and #S10, therefore these tracks were not included in FPT analysis. The larger scale (>100 km) was only detected in longer tracks (# S8, 9 and 18), whereas for all individuals (*n* = 5) both mesoscale and fine-scale changes in movements patterns were detected. The overall peaks identified, between 5 and 8 km and again in between 30 and 70 km, are likely reflecting nested ARS scales. However, in this study we were interested at the fine-scale intense searching and thus, only the smaller scales were considered and assigned to every 12 hours' sunfish positions. For two tracks only speed and sinuosity were considered (#S6 and #S10) whereas for the remaining five all three track metrics were considered in ARS determination (Figure 28).

ARS temporal scales varied between individual fish (Figure S10 for a detailed mapping of the four longest GPS tracks, with ARS segments highlighted in red, travelling movements in black); and depended on the duration of tracks. On average sunfish performed ARS 53% of the time (± 26 % s.d).

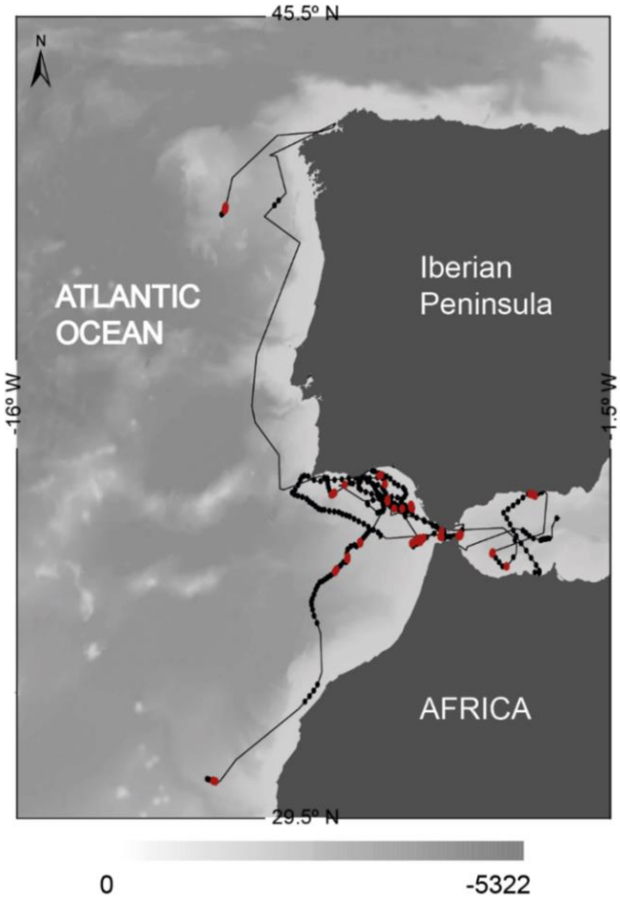

Figure 28 Overall areas of intense searching (ARS - red) detected along sunfish interpolated (black) tracks.

5.4.4.2 Environmental integration of sunfish ARS

Individual sunfish ARS coincided with simulated monthly maximum PD, and a link to higher potential encountered levels of particle (prey) aggregation during foraging was also evident (Figure 29). Daily distances to maximum PD levels, within the 20 km daily displacement radius decreased significantly when sunfish were presumably foraging compared to when travelling (*t* test= 2.5181, *p* <0.05 and df = 235). Hence, a simultaneous high space use of higher simulated PD regions was found when sunfish exhibited intensive movements, characteristic of active searching. Likewise, sunfish in ARS showed a positive (skewed) trend towards SST gradients, whereas similar SSTs and chlorophyll *a* were likely experienced by the tracked fish in both behavioural modes (Figure S 12).

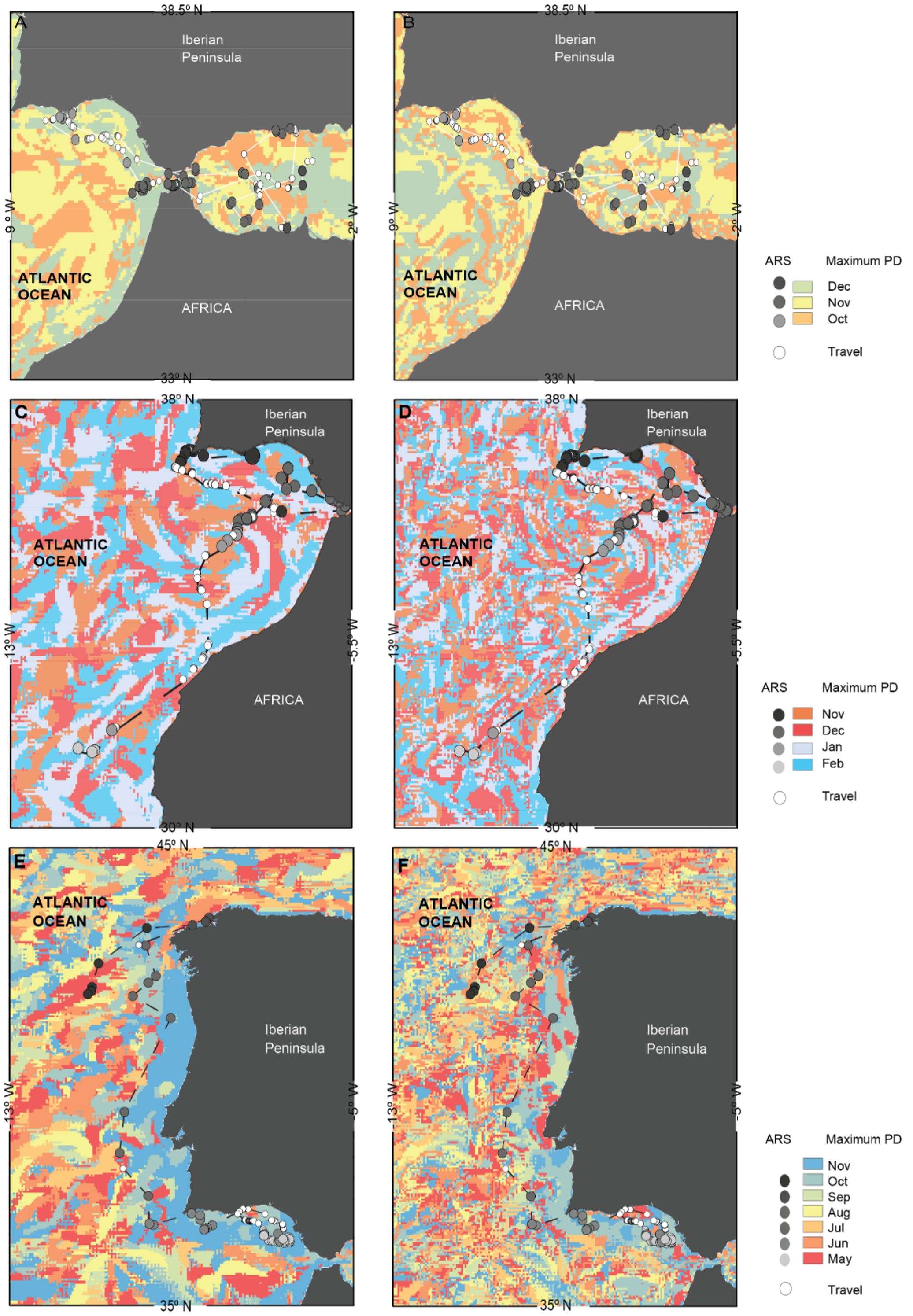

Figure 29 Sunfish tracks coloured by month and behavioural mode (grey scale when in ARS with gradient linking to each month; and white when travelling) superimposed on PD aggregation maps also coloured by the month of maximum simulated PD (A, C and E) and second highest simulated

productive month (B,D and F). Tracks 2013 (#S17 and 18) with gradient of monthly maximum densities on the background (A), second highest productive month (B); Track 2008 (#S8) on monthly maximum densities (C), second highest productive month (D); Track 2010 (#S9) overlaid with gradient of monthly maximum densities (E), second highest productive month (F).

Regarding the GAMM, no correlations higher than 0.75 were found among all four variables prior to the modelling runs, discarding any colinearity amongst predictors in the full model (Zydelis, Lewison et al. 2011). Moreover, an AUC of 0.87 was obtained for the 25% of the dataset used for testing the model, and hence validated the general model framework. Generally, AUC is used to discriminate between true and false positive rates of binomial model predictions and values above 0.7 indicate reasonable model performance (Zydelis, Lewison et al. 2011). With the exception of chlorophyll *a,* the remaining variables significantly predicted sunfish ARS occurrence ($p < 0.05$) (Table S 1). Temporal correlation structure was accounted for by including an autoregressive variance to address the lack of independence inherent to tracking datasets, which was further confirmed by the obtained parameter ρ = 0.786. Comparison of the different models' AIC values revealed that incorporating the temporal correlation structure and excluding the non-significant chlorophyll *a* are both substantial model improvements (Table 18). Sunfish ARS was consistent with an optimum SST between 17º and 22ºC, areas of higher simulated aggregation of buoyant organisms (PD) and strong SST gradients (Figure 30).

Table 18 AIC comparison between the full model including the autocorrelation structure, the same model without the temporal autocorrelation, and the final model with no chlorophyll *a.*

	AIC	**ΔAIC**	**relative likelihood**	**wAIC**	**BIC**	**Log likelihood**	**df**
Full model	772.501	14.933	0.0006	0.0006	808.16	-375.25	11
No autocorrelation	919.099	161.531	8.4e-36	8.39e-36	945.03	-451.55	8
Final model no chlorophyll *a*	757.568	0	1	0.9994	786.74	-369.78	9

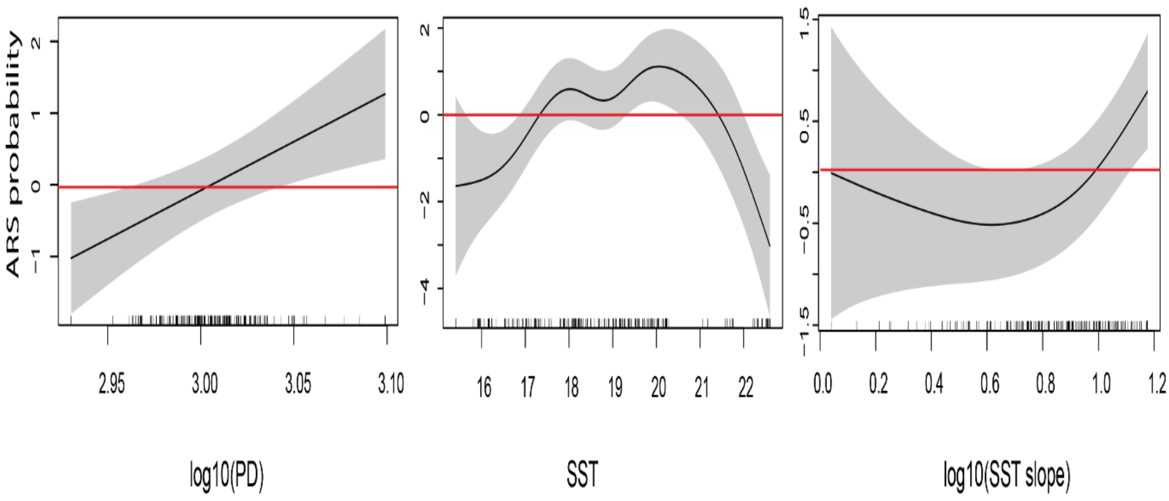

Figure 30 Final GAMM output of each significant variable (Log$_{10}$ simulated PD; SST and Log$_{10}$ of SST gradients) for probability of occurrence of ARS along tracked sunfish movements. Increased simulated PD and gradients of SST together with a thermal optimum [17 – 22ºC] were found to drive sunfish intense foraging behaviour.

Finally, the most parsimonious model excluding chlorophyll *a* and including the autocorrelation structure (

Table 19 for final coefficients output) explained 33% of the variance in sunfish ARS probability of occurrence, with the predicted output resultant from the model significantly correlating with the sunfish behaviour ($r_p = 0.56$, df = 187, $t = 9.199$ and $p < 0.001$). The goodness of fit (GOF) test also confirmed that our model fitted well the behaviour of satellite-tracked sunfish, with no significant difference found between the model and the observed data (Hosmer and Lemeshow test: $p > 0.05$, $\chi^2 = 12.1047$, df = 8).

Table 19 Final model coefficients for the probability of sunfish ARS occurrence as a function of plankton densities, SST, SST gradients.

Predictor	**EDF**	**Ref. df**	**F**	***p value***
Plankton density	1	1	9.649	0.0022
SST	5.064	5.064	6.449	1.37E-05
SST gradients	1.637	1.637	8.789	0.0008

5.5 Discussion

This study investigated sunfish movements and behaviours in relation to simulated prey fields and at different spatial scales. The relative theoretical foraging success in tracked fish movements was estimated from the total encountered model-simulated planktonic species, using a high resolution model of geostrophic currents to identify areas of likely higher plankton densities. Importantly, estimated prey fields were positively correlated with *in situ* measurements of increased abundances of gelatinous species recorded by the Continuous Plankton Recorder (CPR) survey. Firstly, we investigated the large-scale movements of sunfish and found that tracked fish spent more time in areas where the model indicated higher density prey fields would occur. Simultaneously, sunfish paths resulted in greater encounters and consumption of the virtual plankton, larger than 60% of the generated null tracks. Hence, we were able to determine that, if plankton distribution is well defined by the geostrophic currents, sunfish exploited generally higher productivity regions. However, increased simulated densities are mainly encountered by the fish when available habitat is less productive (e.g. during autumn and winter), otherwise real sunfish movements performed no better than random tracks (in elevated simulated plankton densities). Furthermore, GPS tracked fine-scale movements revealed likely foraging (ARS) patterns (≤7 km) and these were also linked to increased simulated productivity. Hence, to our best knowledge we present here for the first time, the analysis on the environmental drivers of likely foraging patterns of the sunfish, confirming an intense use of both areas with steep putative thermal gradients and model estimates of increased abundance of planktonic species.

5.5.1 Plankton densities generation and validation of PD using CPR and SSH remote sensed

In the North Atlantic, the determination and characterisation of plankton ‘hotspots’ was greatly improved by the extensive sampling of the continuous plankton recorder (CPR). However, the area between 30º and 44º N is not intensively sampled and has some long sampling gaps (see Fig. 2 in Richardson, Walne et al. 2006). Hence, for animals inhabiting such southern areas, as our tracked fish, it is still difficult to assess predator prey interactions comprehensively. This lack of co-occurrence of datasets hindered the determination of the relationship between sunfish movements and *in situ* zooplankton measurements. Therefore, a different approach was undertaken by us to estimate the potential prey distribution and to which sunfish trajectories could be compared. Although not strictly influenced only by currents, free-floating plankton are expected to have a close relationship with geostrophic currents (Richardson 2008, McManus and Woodson 2012). In fact, we found a spatial overlap between occurrence data on gelatinous prey (Cnidaria, Siphonophorae and Thaliacea presence) gathered on the CPR routes and the estimated plankton densities from our model (Pearson correlation coefficient 0.400, p <0.001). Hence, the hypothesis that planktonic species' distribution are mainly driven by the currents' velocities was corroborated by the tight relation between modelled PD and the CPR measurements. Importantly, the recorded outbreaks of the scyphomedusa (*Pelagia noctiluca),* off Ireland in October 2007 by the CPR were confirmed by net tows (Licandro et al., 2010, comparing CPR swarms events and records from Doyle et al., 2008). Hence, CPR can provide reliable information to help clarify the regions and periods in which gelatinous plankton prefer to bloom and thus, be a liable validator for the designed prey fields in this study. Importantly, the modelled prey field resolved seasonally persistent oceanographic features known to influence the zooplankton patchiness, for example: (a) in summer, the aggregation of non-migrating planktonic species (Batchelder, Edwards et al. 2002) and larvae (Otero, Álvarez-Salgado et al. 2009) off both western Iberia and north-west Africa that are likely to be caused by the strong upwelling; (b) the European continental shelf edge exhibits gradients of persistent zooplankton with enhanced copepod biomass, especially off southwest of Ireland (Le Fevre 1986); and (c) the onshore transport of planktonic species by the internal tides possibly linked to the upwelling-relaxation event in autumn (Shanks, Morgan et al. 2014). However, further validation tests with drifter buoys and/or tracking of free-floating species are ways in which the present model plankton field could be further validated. Additionally, we also found a close relationship between PD and SSH (an eddy proxy - Chelton, Schlax et al. 2011). Even though productivity ‘hotspots’ are not always detected by remote sensed imagery (Hays, Hobson et al. 2006), the close association of productivity with eddies has been shown in different studies with both mesozooplankton and fish larvae aggregating at the vicinity of these features (e.g. McGillicuddy, Robinson et al. 1998, McGillicuddy, Anderson et al. 2007, Muhling, Beckley et al. 2007). Furthermore, the enhanced productivity within eddies has also been shown to drive the aggregation of higher trophic level species (e.g. Seki, Polovina et al. 2002, Polovina, Balazs et al. 2004, Hays, Hobson et al. 2006, Doyle, Houghton et al., 2008). The positive correlation between maximum absolute values of SSH and PD also suggests that both cyclonic and anti-cyclonic eddies were closely related to our simulated areas of elevated plankton abundance. Therefore, it is likely that our simulated densities represent the general distribution of sunfish potential prey.

Significant logistical difficulties exist when trying to identify and characterise the distribution and abundance patterns of prey upon which marine predators rely (Sims, Witt et al. 2006). Here, unlike other similar studies for which CPR data was available (Sims, Witt et al. 2006, Witt, Broderick et al. 2007) and despite being tentative, the simulated prey fields and the consequent inference of the behavioural patterns adopted by the sunfish have proven valuable where more data-rich alternatives are not available. Given the close relationship between generated PD and SSH (both from the model and remotely sensed albeit at a coarser scale), we suggest that for the cases where no CPR data are available, modelling aggregation ‘hotspots’ based on current data is a useful option. A great advance in studies aiming to investigate predator-prey interactions is the deployment of animal-borne video cameras. Recent results on the direct observations of feeding events by marine predators are promising (e.g. Fossette, Gleiss et al. 2011, Nakamura, Goto et al. 2015). However, the short-term temporal and spatial scale of video-tag deployments still hampers the study of a foraging behaviour profile at the population level.

5.5.2 Real vs. Random PD – Foraging success

We tested the hypothesis that broad-scale movements of sunfish target areas of enhanced plankton densities, by comparing tracked fish trajectories with null locations across the model PD field. Despite the different years of tracking, we found that sunfish movement resulted in an overall higher than average simulated PD encounters. Overall, real sunfish tracks resulted in greater simulated PD encounter than 60% of the null tracks. These results suggest that sunfish movements are structured so as to increase the likelihood of prey encounter when the location of such prey is not completely known and searching becomes necessary (e.g. Viswanathan 1996, Viswanathan, Afanasyev et al. 2001, Benhamou 2007, Humphries, Queiroz et al. 2010, Hays, Bastian et al. 2012, Sims, Humphries et al. 2012). However, this higher than null pattern was not maintained throughout the seasons. Interestingly, sunfish displacement in autumn/winter months was apparently more effective in locating prey-rich areas, with real sunfish tracks outperforming 74% of random (null) tracks in terms of modelled PD encountered. In contrast, warmer months of spring and summer resulted in sunfish exploring less productive (at surface) regions than the average available (performing no better than the null tracks; 54%). This difference likely reflects an area-dependent habitat selection. While the majority of sunfish tracked in warmer seasons (spring/summer) travelled northwards along the Iberian Peninsula, in the colder months of autumn/winter however, sunfish were found returning to the southern Iberia and/or moving either into the Mediterranean or along the northwest African coast. These seasonal movements were associated with frontal areas and sea surface temperature yearly variations (Chapter 3).

In this study, sunfish movements were directly linked to either the Iberian and the NW African upwelling phenomena, which is known to cool surface waters in the region extending up to more than 200 km from the coast, especially during its intense peak in summer months (Relvas, Barton et al. 2007, Miranda, Alves et al. 2012). This oceanographic regime is known for enhancing the productivity in the region and thus fish movements are probably linked with these denser prey fields. Moreover, southern Iberia is part of the Gulf of Cadiz region and is characterised by a strong and relatively permanent frontal area (Stevenson 1977) directly linked to the upwelling regime and variability. Thus, the increased zooplanktonic prey abundance along sunfish summer movements ‘corridors’ may in fact be responsible for the lower estimate of feeding success found, simply because prey was always nearby so was perhaps never limiting at this time. On the contrary, in seasons considered less productive (autumn and winter) sunfish outperformed the *null* tracks, resulting in an increased occupancy of areas of elevated plankton densities when compared to random, presumably because with less prey available overall, finding the prey patches that were present was important. It is known that in areas with less food available and where prey is sparse and unpredictably distributed, predators must adopt movement patterns which will result in increased success rates for foraging (Humphries, Queiroz et al. 2010). As with basking sharks (Sims and Quayle 1998), sunfish movement patterns possibly increase the time spent in the richest prey areas, enhancing prey capture opportunities. Strikingly, the higher than average ‘encountered’ PD found in poorer productive seasons reflect the importance of the behavioural cues sunfish are likely following and that lead to profitable encounters of patches of prey.

5.5.3 Sunfish fine-scale foraging determination and space-use description

An increased space-use of simulated higher PD regions by sunfish when in ARS was revealed, and this overlap was found at both monthly and daily temporal scales. It could be argued that sunfish will also move with the flow and instead of a decision-based movement pattern, fish would passively aggregate in such regions due to a lack of a motor capability. Notwithstanding, sunfish were found to swim against prevailing currents in several different tracking studies (Cartamil and Lowe 2004, Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010) and we also found a size-related rheotactic behaviour for sunfish, with individuals > 0.92m TL swimming frequently facing the direction of major geostrophic currents (Chapter 3). In this study only two GPS tracked individuals were smaller than this threshold and the majority were likely able to swim frequently facing major currents. Overall, at the monthly scale, sunfish ARS movements were found to match peaks in modelled plankton densities; and at the daily scale, the fish position distance towards a maximum modelled density also decreased in ARS defined movement sections. This further supports the idea that sunfish move actively responding to more profitable regions.

The advent of highly accurate Fastloc GPS^{TM} tracking systems, provided robust spatio-temporal species movements' datasets. These have enabled a better understanding of specific behavioural patterns of different marine species, including fine-scale associations between animals and habitat features (Cagnacci, Boitani et al. 2010). The clear improvement of the spatial accuracy of this tracking system was assessed in detail in Chapter 2. Previous studies have applied GPS technology in tracking diverse marine species behaviours, from teleosts, the sunfish (Sims, Queiroz et al. 2009) and broadbill swordfish (Evans, Baer et al. 2011), to marine turtles (Schofield, Bishop et al. 2007, Witt, Åkesson et al. 2010), mammals (Kuhn, Tremblay et al. 2010) and seabirds (Weimerskirch, Pinaud et al. 2007, Votier, Bearhop et al. 2010, Votier, Grecian et al. 2011, Zavalaga, Dell'Omo et al. 2011, Sommerfeld, Kato et al. 2013). Our study is however, to our knowledge, the first to relate the encountered environment to fine-scale behaviours of a teleost species. In the past, GPS tracking of marine animals, mainly mammals and seabirds, have evolved to incorporate depth and other sensors, from which data has better informed us about individual behaviours. For instance, the incorporation of depth-acceleration recorder data was shown to be crucial for non-ARS surface events being eliminated from foraging performance assessments of masked boobies (*Sula dactylatra*)(Sommerfeld, Kato et al. 2013), or to validate ARS detected in northern fur seals (*Callorhinus ursinus*) (Kuhn, Tremblay et al. 2010). Ultimately, new 3D analysis are aimed, as with the Spherical First Passage Time (SFPT) (Bailleul, Lesage et al. 2010), to incorporate both horizontal and vertical dimensions and thus better determine the animals' behaviours in 3D space. Here, we had no access to diving data to perform similar analysis. Feeding event recordings together with accelerometry data are likely to be a simpler attainable solution for sunfish, as previous studies showed with sunfish off Japan (Nakamura, Goto et al. 2015). Nonetheless, the temporal scale of these studies is still short-term, 4–6 days in the later study. In this study, we were able to detect localised residency areas in between faster, straighter movements, with fish exploring the same region for longer times, consistent with previous results (Sims, Queiroz et al. 2009). Moreover, the variable individual ARS times may be a reflection of the encountered habitat differences. Similarly to leatherback turtles (Houghton, Doyle et al., 2006), sunfish may take advantage of patches of prey opportunistically found as they travel.

Species foraging in patchy environments are expected to display ARS behaviour in order to achieve net energy gain, as residency of an animal is likely to increase with habitat profitability (Kareiva and Odell 1987, Barraquand and Benhamou 2008). In fact, at lower prey densities, encounter rates are likely to be sporadic (Fauchald 2009). Although large predators are able to adjust to changes in their habitat with relative ease, planktonic organisms are intrinsically dependent of the balance between behaviour and the environment (McManus and Woodson 2012). Thus, by feeding near the base of the food chain, sunfish are highly dependent on the environmental conditions affecting the distributional patterns of preferred prey. Thus, it appears intuitive that sunfish would be able, as shown here, to respond rapidly and flexibly to stochastic prey availability.

5.5.4 Environmental integration of sunfish ARS behaviour

All sunfish GPS tracked in this study exhibited ARS behaviours in oceanographically diverse areas and despite the broad area occupied, we found sunfish ARS behaviours consistently in areas ~150 km from shore. This conforms to previous studies where sunfish were found to frequently occupy coastal habitats (e.g. Syvaranta, Harrod, et al. 2012, Thys, Ryan et al. 2015, Cartamil and Lowe 2004) and these near shore regions are likely used as nursery, movement corridors or foraging ‘hotspots’ (Block, Jonsen et al. 2011).

The lack of significance of chlorophyll *a* in explaining occurrence of intense searching by sunfish suggests a lagged response of the species to primary productivity. This result is expected because sunfish are mainly feeding on zooplanktivorous organisms, either gelatinous or small crustaceans (Syvaranta, Harrod et al. 2012, Nakamura and Sato 2014, Chapter 4), which then feed at the bottom of the food chain. This mismatch in timing between phytoplankton (chlorophyll *a*) and zooplankton is a well-known relationship between the resource supply and consumer demand (Thackeray 2012). In fact, this disparity between both assemblages is well known (e.g. Rossi, Sabatés et al. 2006), with the mismatch between predators and chlorophyll *a* already described for various species including whale sharks (Sleeman, Meekan et al. 2010) and more recently in this study for sunfish broad-movements and habitat selection in the same geographic region (Chapter 3).

Furthermore, SST was found to have a significant influence with the description of a narrow optimum of ~5ºC range (from 17 to 22ºC) for ARS to occur along sunfish paths. In previous studies, SST has been suggested to be a main driver for sunfish coarse-scale distributional patterns, influencing major migratory movements of the species in both eastern and western North Atlantic (Sims, Queiroz et al. 2009, Potter, Galuardi et al. 2010) and western and eastern Pacific (Cartamil and Lowe 2004, Dewar, Thys et al. 2010). In this study, a restricted thermal envelope was found to influence ARS behaviour of tracked individuals, rather suggesting sunfish feeding habits in the north-east Atlantic are consistent with a narrower preferred thermal range than previously realised. Thus, here SST is characterised as an important driver for ARS behaviours of sunfish, further supporting previous results related to sunfish habitat selection (Chapter 3). Generally, SST gradients are a proxy for frontal regions, areas of significant physical and biological activity and are characterised by the convergence of different water mass types (see Scales, Miller et al. 2014 for a thorough review). Increased permanence of these features within the same geographic area increases the concentration of both phytoplankton and zooplankton, which consequently attracts higher trophic organisms (Prants, Budyansky et al. 2014). All these major regions as well as smaller-scale frontal areas have been highlighted to be associated with increased permanence for sunfish. For instance, in previous studies in the western English Channel sunfish were mostly associated with frontal water masses (Sims and Southall 2002); and in the southern California Current System a close link was recently seen between sunfish migratory paths and upwelling-driven frontal regions

(Thys, Ryan et al. 2015). Similarly in the western Iberia sunfish habitat selection was found to be consisted with frontal areas (Chapter 3).

Lastly, sunfish ARS in this study was largely associated with increased simulated plankton densities index (PD), which through simulations identified areas of aggregation of free-floating organisms. Here, we focused on the high resolution movements and behavioural patterns, finding a close link of active searches and areas of likely accumulation of known sunfish prey items. Sunfish appeared to exhibit a controlled response to dense prey patches encountered along real fish movements, indicated by the decreased proximity to maximum peaks in PD when in ARS, which infers sunfish actively respond to encountered prey distributions by structuring movement patterns across a rnage of scales and differently through the changing seasons.

5.5.5 Final conclusions

This study provides an important step forward in understanding the ecological drivers for the behaviour of the largest teleost fish, the ocean sunfish. Here, we described the relationship between predator behaviours and concurrent spatio-temporal biological activity and our findings have implications for the detection and interpretation of predator-prey interaction simulations. We have provided both visual and statistical evidence that sunfish area restricted search behaviour is strongly associated with areas of likely accumulation of weaker swimmer organisms, either driven by sea surface temperature gradients or by current shear, and all within a narrow thermal envelope. Ultimately, the relationship we found for areas of accumulation of floating organisms and the planktivorous ocean sunfish can be further explored and validated with studies of other marine predators.

Chapter 6: Spatial and temporal variation of sunfish susceptibility to longline fisheries in the north-east Atlantic

6.1 Abstract

The susceptibility of the vulnerable ocean sunfish (*Mola mola*) to longline fisheries has not yet been assessed elsewhere, despite the high level of recorded bycatch in this and other fishing activities. The low commercial value of this species, being only consumed in relatively few places in the World, has probably underlined the lack of understanding of the extent of susceptibility to fisheries activities. Using data from 18 sunfish satellite tracked since 2007 and VMS data from both the Portuguese and Spanish pelagic longlining fleets, this study identified a positive correlation between areas of high spatial sunfish occupancy and those of intense activity by the two fishing fleets analysed, in the north-east Atlantic. As much as 56% of the regions occupied by the sunfish were exploited by the fishing vessels. Moreover, we found a seasonally differential

spatial overlap with increased shared occupancy in both spring and summer months. From the estimated interactions (co-occurrence of fish and boats in the same grid cell at the same time) tracked sunfish were at risk from longline capture, on average, two days per month. Analysis of the seasonality of these interactions revealed that spring is the season with highest risk whereas winter presented the lower risk. Analysis of depth occupancy revealed sunfish occupy the top 200 m at night for as much as 95% of the time, matching temporally the hook deployment depths. The lack of compulsory reports on this species hinders the assessment of the susceptibility of the world's largest bony fish to fishing activities, although its high incidental capture worldwide indicates a need for susceptibility assessments. Although only 18 individuals were tracked, our results showing high species and vessel co-occurrence emphasises the need to consider its conservation and potential bycatch mitigation measures.

6.2 Background

Obtaining precise information on the migratory patterns, distribution, behaviour, foraging ecology and residency of pelagic predators has been hindered by the broad geographic area occupied by the majority of species and the applicability of tracking technology. Indeed, along-track continuous monitoring of a migrant is difficult, especially in species that surface briefly (Gurarie, Andrews et al. 2009). However, over the past decades, and especially in the marine environment, enhanced techniques, such as satellite-relay electronic tags, have been developed to reveal habitat selection and movement dynamics of numerous species. Such satellite tracking technologies have increased our knowledge of population dynamics and individual behaviour of numerous oceanic species (e.g. Weimerskirch and Robertson 1994, Sedberry and Loefer 2001, Block, Costa et al. 2003, Weng, Castilho et al. 2005, Mansfield, Saba et al. 2009, Sippel, Holdsworth et al. 2011, Queiroz, Humphries et al. 2012).

Different tracking systems have been developed, including Argos transmitters that enable animals positions to be located by polar-orbiting satellites, with reported errors not exceeding 12 km (e.g. Vincent, MCConnell et al. 2002, Costa, Robinson et al. 2010, Patterson, McConnell et al. 2010,), to pop-off satellite archival transmitter (PSAT) tags capable of recording both temperature and depth experienced by the individual, with inherent spatial errors of ~100 km (Sippel, Holdsworth et al. 2011). Simultaneously, the availability of remote sensed data on oceanographic variables has improved significantly which, coupled with tracking systems enable better understanding of the environmental cues to which species are likely to respond. The combination of such datasets has supported the predictions of habitat selection by marine predators (e.g. Sims, Witt et al. 2006, Block, Jonsen et al., 2011). Ultimately, this will improve our knowledge of how animals might respond to changing conditions in a highly dynamic ecosystem such as the marine environment (Papastamatiou, Meyer et al. 2013).

Numerous marine species frequently inhabit for long periods of time more coastal habitats. In fact, these near shore regions are frequently used as nursery areas, foraging hotspots or movement corridors (Block, Jonsen et al. 2011). Of these, the eastern coastal boundaries are often one of the richest zones of marine biodiversity globally, due to the interface between cold, nutrient-enriched upwelled waters and warmer oligotrophic open water that leads to an elevated productivity (Scales, Miller et al. 2014). Increased productivity, which attracts numerous higher trophic species, however, is probably driving the increased intense fishing effort in these regions (Stewart, Lewison et al. 2010).

Worldwide we are witnessing a significant decline of fish stocks, due to global industrialised fisheries, with at least half of the stocks being either fully exploited or overexploited (Worm, Hilborn et al. 2009). Marine ecosystems are severely affected by fishing activities which has led to the recent increased interest on the environmental impact of such activities (Votier, Bearhop et al. 2010). The evaluation of the impact of fisheries on pelagic organisms presents three major problems: (a) the time required to detect population changes of long-lived organisms, (b) the existence of sublethal effects and (c) the challenges associated with surveys of pelagic organisms (Lewison, Crowder et al. 2004). One of the major threats of fishing activities is the removal of non-targeted species (bycatch) which is known to ultimately affect the ecosystem and of which little information is available globally (see Lewison, Crowder et al. 2004 for more detail). Unregulated bycatch can result in changes in biodiversity by altering species evenness, whether increasing or decreasing it (Zhou, Smith et al. 2010). The removal of predators may also lead to trophic cascades in the ecosystem (Carpenter, Kitchell et al. 1985). Thus an ecosystem-based approach for marine conservation comprises the protection of all trophic levels, including top trophic level species (Louzao, Pinaud et al. 2011). Although identified as a fundamental problem for species and ecosystem dynamics, global bycatch assessments are still hampered by the inconsistent and imprecise approach to recognise locations, timing and environmental conditions (Rogan and Mackey 2007, Zydelis, Lewison et al. 2011, Clarke, Sato et al. 2014, Roe, Morreale et al. 2014). Furthermore, effects of bycatch across fishing gears at the population level have only been recently recognised (Wallace, Kot et al. 2013, Lewison, Crowder et al. 2014).

The better understanding of the habitats occupied by marine species has been aided by the environmental integration of satellite tracked movements (e.g. Block, Costa et al. 2003, Damalas, Megalofonou et al. 2007, Walli, Teo et al. 2009), revealing important ‘hotspots’. It is known that marine predators tend to aggregate forming biodiversity hotspots in the ocean (Worm, Lotze et al. 2003). This, in turn, may drive increased vessel efforts in these focal areas with high attendant numbers of catches and bycatch rates. Despite its worldwide low commercial value [with the exception of Japan (Watanabe and Sato 2008) and Taiwan (Liu, Lee et al. 2009)], ocean sunfish are a major bycatch component in several different fishing activities, including longlining (Damalas, Megalofonou et al. 2007, Peterson 2007) and driftnets (Silvani, Gazo et al. 1999). Essentially driven by the increasing reported rates of bycatch, there has been an increased interest in sunfish conservation assessments (Fulling, Fertl et al. 2007).

Recently, the IUCN Red List of Threatened Species committee re-evaluated sunfish which is now *Vulnerable* due to the high risk of extinction as a consequence of the increased levels of captures reported worldwide (Jing, Zapfe et al. 2015). Critically, sunfish populations are decreasing. Although not being a commercially important species in the study region of the Gulf of Cadiz, analysis of sunfish interactions with fisheries is of great value for future conservation measures, at both the species and ecosystems levels. In addition to captures in nets, sunfish are also captured on pelagic longlining baited hooks (GRM *personal observation*), being lately discarded without a precise injury assessment. Hence, the aim of this study was to assess qualitatively the potential susceptibility of sunfish to longlining activities in the north-east Atlantic. Ultimately, information on sunfish vulnerability to fisheries can also be relevant to many other species to better inform management measures.

6.3 Methods

6.3.1 Tagging and tracks processing

Between February 2007 and October 2013 we successfully tracked 18 sunfish with three different tag models: PSATs (PAT-MK10, Wildlife Computers); SPOT5 Argos Platform Terminal Transmitter (Wildlife Computers) and an integrated Fastloc^{TM} GPS (Wildlife Computers and Sirtrack Ltd). Tagging and the subsequent track processing, including filtering for erroneous positions, geolocation of light-levels and interpolation methodology were all described in Chapter 3.

Most probable daily tracks were plotted using ArcGIS geographical information system (ESRI Inc., CA, USA), where the home range (95% minimum convex polygon, MCP) was estimated. The total time spent within each 1 x 1º cell (termed occupancy) was computed by summing the number of daily corrected points located within cells. Spatial usage from all individuals was then investigated by means of kernel density estimator (KDE) function in ArcGIS. To reduce tagging area bias effects, number of positions per grid cell was normalised by dividing it by the number of individual sunfish within each grid cell (Walli et al. 2009). Final number of mean days per grid cell was then an effort-weighted index of residence per unit area. Seasonal analysis of all tracks combined was also performed with seasons defined as follows: winter (December – February); spring (March – May); summer (June – August) and autumn (September – November).

Lastly, PSAT tracked depth data was explored to determine the vertical occupancy of sunfish, including the diel variation and in relation to known longliner hook deployment depths. Data from time bins encompassing either sunrise or sunset hours were excluded from the analysis, assuring no night hours were considered in daylight period or *vice versa*. Diel periods were split following the algorithm provided by the National Oceanic & Atmospheric Administration (NOAA)[8].

6.3.2 VMS dataset from two fleets' analysis – collection and filtering

Data from both complete pelagic longlining fleets from Spain and Portugal were obtained from respective national fishing monitoring centres. In total, we analysed 186 vessels' GPS position data, time stamp and anonymous identification number, since no access to registration number or name, dimensions or administrative port was granted. The Spanish dataset spanned from January 2005 to December 2009 and comprised data every two hours; whereas the Portuguese data was collected at every hour and ranged from January 2003 to December 2005 and January 2009 to December 2011. For both datasets, an algorithm was developed to detect turning angles during fishing activity (>130º). In addition, distance travelled up to and from the turning point was calculated to determine the deployment starting and ending positions, also considering the longline length (approx. 100 km). To retain only fishing positions, all GPS movements between these locations were cleaned from the dataset, including trips to and from landing docks. Fishing data were converted into daily positions, by calculating the daily centroid from each fishing cluster comprising the first and last positions.

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[8] http://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html

Fleet nationality was ignored in the analysis due to the mismatch in the temporal scale of both datasets and all possible fishing scenarios were accounted by combining annual datasets. Thus, a total of 30 different fishing scenarios, where all yearly Spanish (n=5) and Portuguese (n= 6) datasets were individually combined, were analysed. Vessels' daily space use was also analysed by means of a kernel density estimate (KDE) in ArcGIS, following the same procedure applied to sunfish tracks.

6.3.3 Overlap coefficient (KDE)

To test for significant associations between both species and vessel space use patterns, a Pearson correlation was performed, estimating a statistical relationship among the environment selected by both fish and fishing vessels. Both fish and vessel estimated occupancies (KDEs) were multiplied to assess the combined space use in each grid cell. This analysis was subsequently repeated per season.

6.3.4 Interactions coefficient

Sunfish theoretical vulnerability to longliners operating in the region was further assessed using custom-written software (*Grid Occupancy – MBA*). Here, corrected daily locations of sunfish and daily centroids of fishing boats were gridded into 1º grid cells and simultaneous presence (counts; occupancy density estimation) were computed. Interactions between tracked fish and vessels were then computed per fishing scenario by calculating the number of days-at-risk (simultaneous occupancy). Subsequently, to test whether the observed fishing pressure differed significantly from the 'risk' facing random walk sunfish tracks, we also calculated the mean number of days-at-risk for simulated null fish (n=300 random trajectories per tracked path), using both step-lengths and turning angle distributions computed from real tracks, that was similar to the simulation described in Chapter 3. Fishing locations overlapping with both real and random tracks were compared using Mann-Whitney *U*-test; $p < 0.05$.

6.4 Results

6.4.1 Spatial distribution

A general preference of tracked sunfish for the Gulf of Cadiz (GoC) region is evident (Figure 31 C). However, seasonal patterns emerged. During winter, a higher fidelity to southern latitudes off the north-west African coast or the Alboran gyre in the Mediterranean was observed (Figure 32 A); whereas in spring sunfish aggregated in the GoC (Figure 32 B); in summer months sunfish occupied northern latitudes (Figure 32 C); lastly, a preference for southern regions was again detected in autumn (Figure 32 D).

Similarly, overall, fishing activity focused in two main areas (1) western Iberia and (2) Gulf of Cadiz (Figure 31 D). During winter the GoC was generally occupied by fishing vessels (Figure 33 A), whereas high fishing effort was observed in both main areas during spring and autumn (Figure 33 B and D). Although patchily distributed, boats in summer tend to concentrate off the western Iberian coast (Figure 33 C). Globally, the study region defined by the sunfish MCP appears to be most heavily fished in autumn and winter.

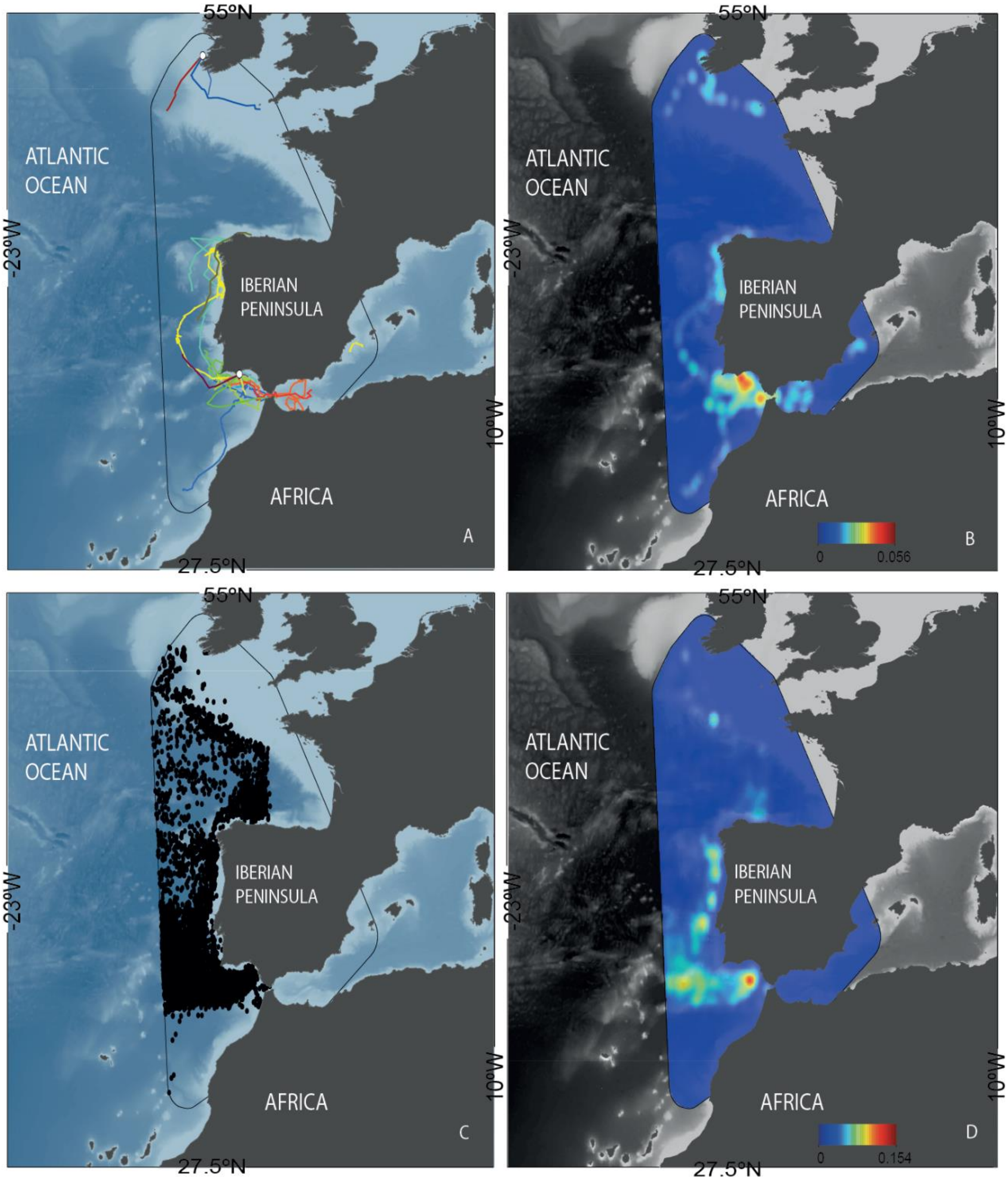

Figure 31 (A) Sunfish tracked in this study, refer to Chapter 3 for more detailed track analysis; (B) KDE of high intense usage areas for those tracked sunfish revealing increased occupancies In the GoC, NW Iberia and the Gibraltar/Alborean sea; (C) pelagic longliners VMS daily centroid position constrained to the sunfish calculated 95% MCP; (D) surface longliners space use depicted by KDE with both the GoC and SW Iberia highlighted.

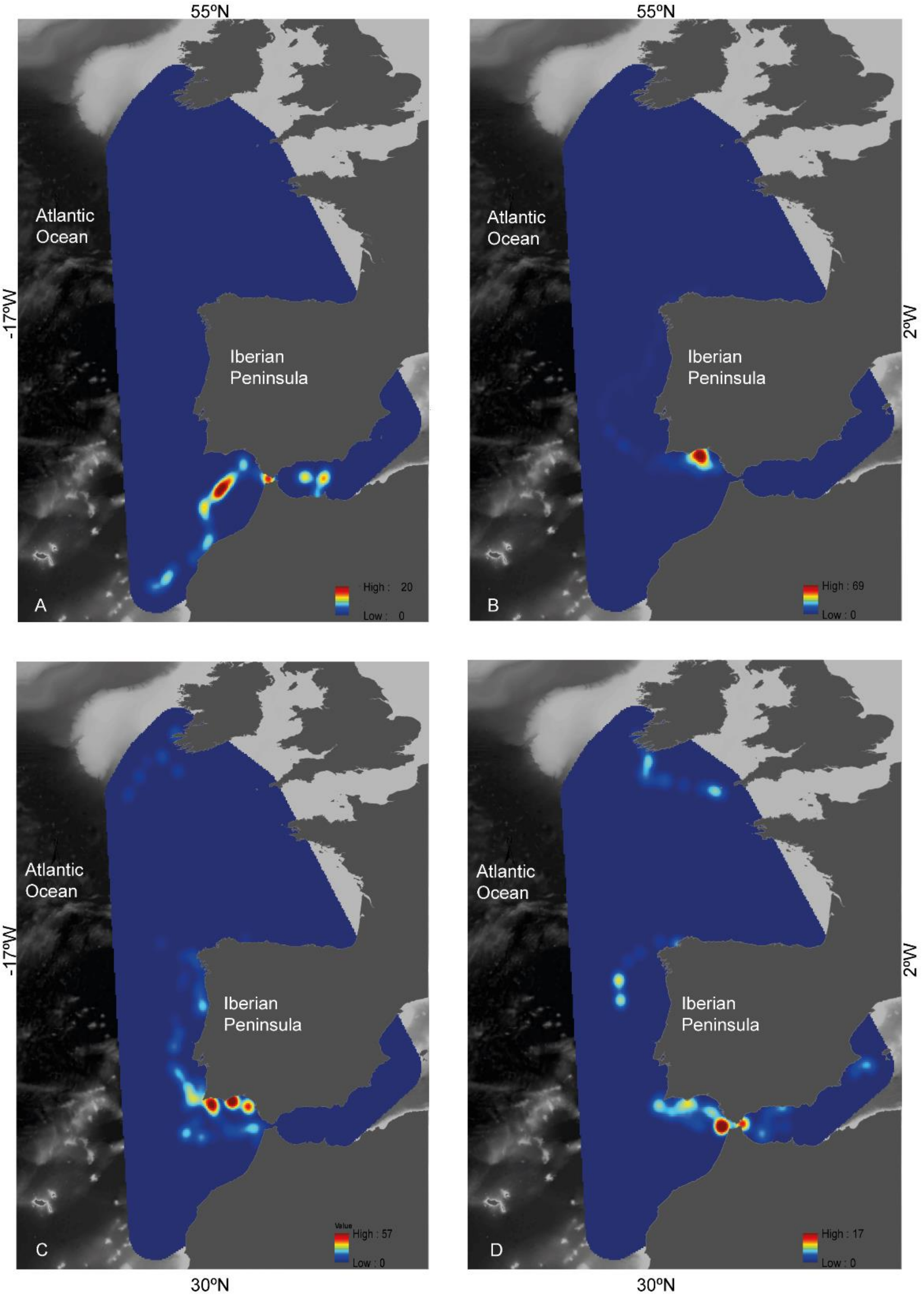

Figure 32 Sunfish seasonal space use estimated (KDE - mean days per grid cell) A) winter with southern occupancy (GoC, NW Africa and Alboran sea); B) spring GoC higher residency for sunfish; C) summer the northwards migration along the western Iberia is highlighted and D) autumn sunfish occupancy at northern latitudes and the Gibraltar region.

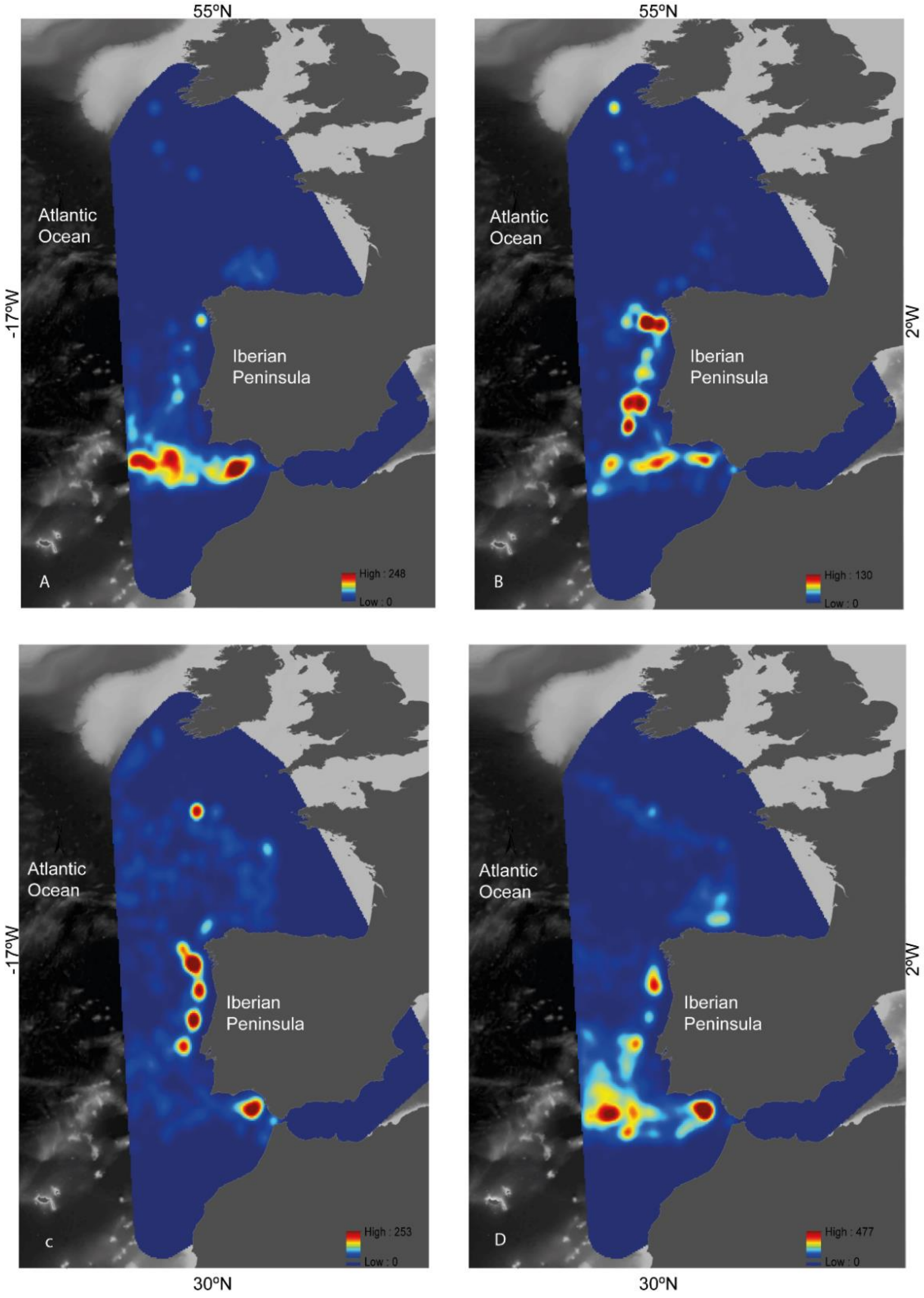

Figure 33 Surface longliners seasonal space-use KDE: A) GoC and SW Iberia intensive activity during winter months; B) all coastal region off Iberia intensively surveyed in spring ; C) summer fishing activities concentrated GoC, NW Iberia and less spread around the 95% MCP ; D) autumn with increased efforts SW Iberia and GoC and some in the NW of the Peninsula.

6.4.2 Susceptibility of sunfish to longlining

A positive correlation was found between sunfish distribution and vessels' spatial occupancy at 1º grid cells, within the sunfish 95% MCP ($r_p = 0.251$, $p<0.001$). Hence, we repeated the same analysis quarterly to investigate possible seasonal patterns of susceptibility to surface longlining (see Figure 34). Interestingly, increased correlations were found both in summer and spring months, reflecting an increased fishing effort within fish occupancy during these seasons (Figure 34).

Resultant overlap between sunfish and longliners KDEs showed a clear seasonal differentiation in the total space occupied (Figure 34). Overall, the combined use between both fishing fleets and sunfish density estimates was clearly focused on the GoC, the entrance of the Gibraltar strait and in northwest coastal Iberia (Figure 34 A). This analysis revealed that the VMS space use overlapped with as much as 56% of the fish distribution (estimated for the tracked individuals).

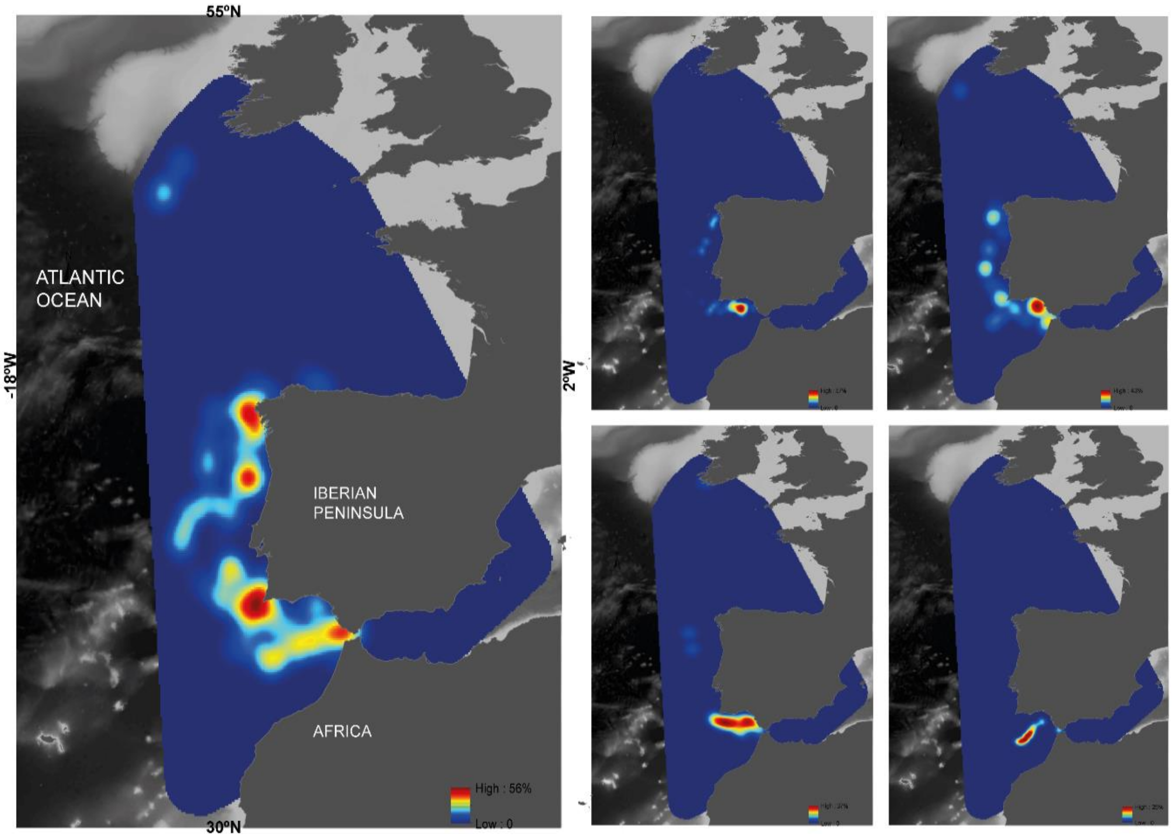

Figure 34 Overlap between both sunfish and fishing fleets distributional patterns. A) spring; B)

summer; C) autumn; D) winter. Colours are depicting where high overlap is found and % of co-occurrence is given individually.

Table 20 Seasonal correlation coefficients (Pearson) for simultaneous space-use between sunfish and longliners preferred areas of occupancy.

	Autumn	Winter	Summer	Spring
r_p	0.13	0.09	0.24	0.29
p	<0.001	<0.001	<0.001	<0.001
% Overlap	37	25	43	57

Seasonally, increased overlap is maintained at 57% during spring months being mainly restricted to the GoC and western Iberia regions, whereas in summer months longliners occupy 43% of the sunfish distribution along the western Iberia upwelling region. In autumn, 37% of sunfish distribution coincided with fishing vessels' focal areas off Gibraltar and off northwest Iberian coast. Lastly, a sharp reduction to 25% of fish space-use overlapped with longliners during winter months. Interestingly, overlap between fish and longliners was decreased in the seasons with higher fishing intensity in the region (Table 20).

6.4.3 Fish-vessels interactions

Interactions between sunfish movements and fisheries in space and time were calculated as a function of annual activity and were independent of the number of vessels operating. There was no significant correlation found between encounters and number of vessels per day ($r_p = 0.030$, $p = 0.124$). Overall, sunfish spent on average 2.03 (± 1.44 s.d.) days-at-risk per month, with this index reaching a maximum of 4.5 days per month. Importantly, detected risk for real sunfish trajectories was significantly greater than the mean number of days-at-risk found for the null (n = 300, median = 0.58 days per month) sunfish paths (Mann-Whitney = 258.0, p =0.0184).

The susceptibility of sunfish to longlines was not equal throughout the year, instead being significantly different across seasons (Anova F = 4.29, df =3 and *p* <0.05). Overall, sunfish spent a maximum of 4.0 days at risk per month during spring, roughly half of that time during both summer and winter periods, and a maximum of 3.0 days per month in autumn (

Table 21).

Table 21 Days at risk per month seasonally

	Summer	**Spring**	**Autumn**	**Winter**
Average	0.378	1.109	0.426	0.104
Max	1.916	4.077	2.581	1.871
Min	0.000	0.000	0.000	0.000
Sd	0.623	1.322	0.861	0.441

6.4.4 Sunfish depth use in relation to longlining vertical deployment

To determine the extent of overlap between sunfish and deployed hooks at depth, the vertical occupancy of sunfish in the water column was analysed (Figure 35). Sunfish spent a considerable amount of time at the same depth as the deployed hooks, denoted by the grey bar, and this is increased during the night, thus matching the hours of fisheries' activity. Importantly, all nine individuals for which depth records were available, spent as much as 90% of the time between the surface and 200 m, increasing the chances of a baited hook encounter.

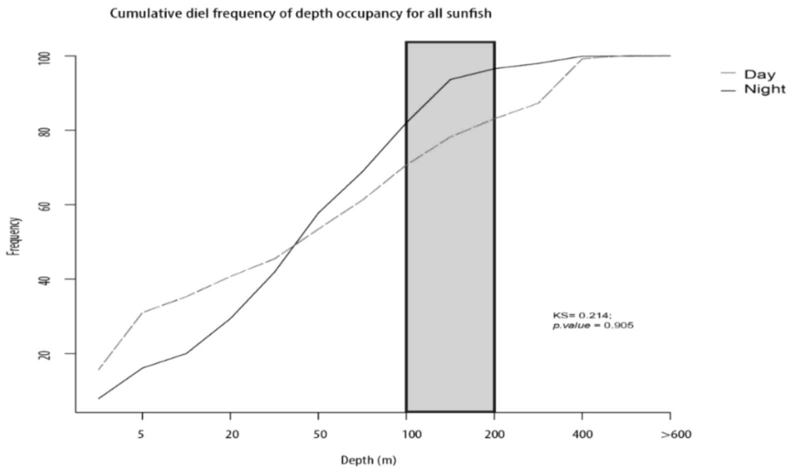

Figure 35 Sunfish day (grey) and night (black) overall cumulative depth occupancy in relation to the longlining vertical exploitation (grey vertical bar).

6.5 Discussion

Despite of the lack of consistent reported captures of ocean sunfish in fisheries worldwide conservation measures are needed given the extremely high values of this species captures when incidental catch is described. In this study, we found a positive relationship between areas of preferred activity of longliners operating in the north-east Atlantic and regions occupied by tracked fish, with this overlap being maintained throughout the year. Overall, simultaneous space use co-occurrence reached as much as 56% of the sunfish distribution, with an estimated susceptibility of ~2 days-at-risk per month for tracked fish. Importantly, sunfish vulnerability was higher than for random (null) tracks indicating that the habitats selected by sunfish were also selected by longliners. In addition, sunfish spent 95% of the night-time periods between 0-200 m, matching the depths at which longliners' hooks are deployed (Ward and Myers 2005). Therefore, by satellite tracking sunfish and analysing VMS data of pelagic longline fleets operating in the north-east Atlantic, we report here a significant shared occupancy of both fish and vessels.

6.5.1 Fishery impact on sunfish

Sunfish are only an important resource in Japan and Taiwan (Dewar, Thys et al. 2010), yet, this species is incidentally taken in large numbers from fisheries globally. Longlining is a worldwide fishing activity which targets one or few species, however, accidental captures of many other species is frequent. This fishery has great impact on sunfish populations off South Africa, where extremely high rates of bycatch were reported (Petersen 2005). Consequently, the assessment of the extent of the vulnerability of the species to longlines remains unclear. Other fishing methods also impact sunfish worldwide, with for example, 51% of bycatch of mid water trawlers in South African waters being found to be sunfish (Peterson and McDonell 2007). In the Mediterranean sunfish were found to comprise as much as 93% of the drift gillnet fisheries non target captures (Silvani, Gazo et al. 1999), whereas sunfish made up ~23 % of the total catch in swordfish fisheries off California (Cartamil and Lowe 2004). Importantly, even though sunfish are often discarded alive, there is no actual data on the post-catch survival rate. In a recent study it was suggested that at least ~ 40% of sunfish captured incidentally in California's driftnet and gillnet fisheries are released injured (Thys, Ryan et al. 2015). In the north-east Atlantic no records or estimates have been taken for the species, hampering comparable assessments. However, given the extent of the overlap quantified in this study, it is likely that similar scenarios may occur in the region.

6.5.2 Sunfish-vessel overlap and interactions

Our results demonstrate that both the Gulf of Cadiz and the western Iberian regions appear to be highly utilised by both vessels and sunfish throughout the years. These regions are known productivity ‘hotspots’ due to intense upwelling phenomena and consequent frontal features that attract and support an entire trophic chain (Relvas, Barton et al. 2007, Scales, Miller et al. 2014). Furthermore, the intense exploitation of frontal areas by longliners has been determined, with vessels known to use SST remote-sensing images to find the regions recognised for the aggregation of target species (Ward and Myers 2005). In fact, the tracking of frontal regions by fishing vessels has already been described in the area (Queiroz, Humphries et al. 2012). Thus, increased persistence of sunfish at the vicinity of frontal boundaries (described in detail in Chapter 3) is likely to promote this species vulnerability to fisheries.

Generally, sunfish seasonal distribution patterns are reflected in the seasonal differential susceptibility to fisheries. During winter months while sunfish move towards southern latitudes, the efforts of some components of the longline fishing fleets are restricted to coastal areas, resulting in the smallest horizontal overlap (also reflected by the weaker spatial correlation) and the lowest encounter rates (days-at-risk per month). Here, it should be noted that sunfish in winter besides moving towards the north-west coast of Africa, also engage in a displacement towards the Mediterranean, (where no fishing data were available in this study).

The simultaneous spatial occupancy is preserved in spring and summer months, with sunfish moving northwards along the Iberian Peninsula and fishing vessels also focusing in the region. In late spring and summer, western Iberia is characterised by a strong upwelling (Relvas, Barton et al. 2007), where the cold productive waters attract higher trophic level predators that aggregate in frontal regions (Walli, Teo et al. 2009, Scales, Miller et al. 2014). Our analysis revealed spring months to be the season with higher spatial overlap and a concomitant increase in the number of days-at-risk per month for sunfish. This result likely reflects the simultaneous local and coastal exploitation within the productive waters of the Gulf of Cadiz. This region has been shown to be a ‘hotspot’ for the species, at least for the juvenile stages (see Chapter 3), and the spatial fishing effort in the area is probably driven by the ‘productivity tracking’ by vessels. On the other hand, in summer the % overlap is still above 40%, although the encounter rates are decreased to half of that found in spring. During this warmer season, probably linked to improved weather conditions, longlining vessels displace further from the region, concentrating the fishing efforts in open waters of the North Atlantic. Hence, this wider spatial distribution probably represents a diminished risk for sunfish, despite the simultaneous occupancy being maintained relatively high. Although similar interaction indices were found for autumn and summer months, autumn presented a decreased overlap percentage of summer periods. During these months, fisheries tend to perform similarly to spring whereas sunfish are more focused towards southern latitudes.

Taken together, seasonal sunfish and longliners spatial distribution suggest that both fish and vessels are probably responding to similar environmental conditions rather than an increased co-occurrence due to chance. Importantly, our study successfully described specific areas and seasons where bycatch susceptibility is high. Without the legal regulations of landing reports, sunfish bycatch records are not made, which impairs our efforts to quantify the actual impact of fisheries in sunfish.

6.5.3 Vulnerability at depth

Survival rates of sunfish released from longlines remains uncertain, despite the records of the incidental sunfish captures throughout the year on longliners operating in the North Atlantic (G. Mucientes., personal observation). Hence, our findings of a shared spatial distribution in the region reflect the extension of the potential threat to which the species is submitted. In addition, sunfish night-time depth occupancy revealed that the species spends as much as 95% of the time in the top 200m, which increases the likelihood of encounters with longliners' hooks (Ward and Myers 2005) by co-occurring at the same water column depth. The analysis of both horizontal and vertical dimensions has been informative for other predators, for example the overlap of blue shark (*Prionace glauca*) nocturnal depths and longlining hooks being reported to exceed 76% in the same geographic region (Queiroz *et al.* 2012). Further similar studies should address the concurrent spatio-temporal usage between vessels and species, as the identification of the vertical usage of a marine species can be used to inform possible bycatch mitigation measures, such as the longline setting at different depths from those occupied by the species (e.g. Bromhead, Clarke et al. 2012). In fact, sunfish bycatch was estimated to be considerably reduced (20 to 25%) by changing the net depth at which drift gillnets are set in California (Cartamil and Lowe 2004).

6.5.4 Importance of the study

Above all, a better description of areas of persistent high interaction rates between species distribution and fishing operations is required for the implementation of adequate protection measures of marine megafauna selecting those habitats. In the European Union, all vessels with lengths greater than 12 metres are required to transmit their GPS locations, at intervals of up to 2 hours, which provides descriptive patterns of fisheries activity with good temporal and spatial coverage (Witt and Godley 2007). To develop active management and conservation plans to reduce bycatch events, the distribution and depth preferences of both target or bycatch species should be determined (e.g. Ward, Lawrence et al. 2008, Lewison, Crowder et al. 2004) which together with the frequency of interactions can better inform fishing operations and practices adjustment (Melvin, Parrish et al. 2001). Area and seasonal closures are management tools that can prevent bycatch hotspots from being over-exploited. However, such closures often result in the reduction of the catch of target species, making them uneconomical (Goodyear 1999). On the other hand, the implementation of high seas MPAs is far from straightforward (Game, Grantham et al. 2009), especially in areas heavily exploited by different nations. Coordinated actions by international parties are required for the effective implementation of conservation measures and bycatch reduction strategies (Lewison, Crowder et al. 2004, Fossette, Witt et al. 2014). Concerted efforts for mitigation effects should include the return of uninjured bycatch. For example, in the Californian drift gillnet fishery, despite showing clear signs of fishery-induced trauma, sunfish is thought to be released alive (Cartamil and Lowe 2004). Thus, the release of captured animals may significantly contribute to sustainable use of fisheries resources by reducing the mortalities associated with vessels operating in areas where most likely sunfish is captured.

An important and informative approach to better understand the bycatch susceptibility is the mapping of the spatio-temporal extent of interactions of species with fisheries (Zydelis, Lewison et al. 2011). Bycatch assessments of seabirds on longliners have contributed with bounded estimates of the impact of fisheries at a likely population-level. For example, bycatch from a basin-wide pelagic longline fishery was quantified for a vulnerable seabird, the Black-footed Albatross (*Phoebastria nigripes*) in the central North Pacific (Lewison and Crowder 2003); or in the Southern Ocean the bycatch and bycatch rates of seabirds are discussed in the context of longliners operations (Tuck, Polacheck et al., 2003). Importantly, the coincident habitats occupied by sunfish and other marine predators, such as the endangered leatherback turtles (Hays, Farquhar et al. 2009) or basking sharks (Sims and Southall 2002) re-enforces the need for an effective reduction of fishing effort in these high space-use areas. Recently, different focal regions in the North Atlantic and Pacific Ocean were identified as highly susceptible for the capture of leatherback turtles (Fossette, Witt et al. 2014, Roe, Morreale et al. 2014). However, such analysis based on data on combined effort and catch statistics with a spatial resolution of 5x5º and over large temporal scales, would greatly benefit from the finer spatiotemporal scale of the fisheries dataset analysed here. By using fisheries data at such large scales, much of the finer-scale information on the species behaviour is lost (Roe et al. 2014). Importantly, high spatial resolution VMS data from all fleets should be made freely available internationally. Notwithstanding, these studies provide information on the potential interaction between species and fisheries activity to inform critical hotspots for conservation measures implementation.

Satellite tracking of sunfish has been proven valuable in revealing important traits of this poorly known species worldwide (Cartamil and Lowe 2004, Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010, Thys, Ryan et al. 2015), however few studies have assessed the real threat fisheries may pose to sunfish (Silvani, Gazo et al. 1999, Tudela, Kai Kai et al. 2005, Peterson 2007). Thus, our study, with the caveat of no precise information on captured fish being available, would greatly benefit from more precise abundance proxies, such as CPUE. Moreover, although clear patterns were found in our study, such as the seasonality in the distribution and susceptibility of sunfish to fisheries, these were gathered by tracking few individuals. Thus, the assessment would greatly benefit from further tagging and tracking of an increased sample size.

Our study did not aim to determine the actual bycatch values of sunfish, instead we present here a scenario-driven exercise to understand the risk of longline fisheries to the World's largest bony fish. Given the lack of actual bycatch measurements, our results have importance in the understanding possible exploitation rates of a species recently listed as *Vulnerable* by the Red List of Threatened Species. Ideally, further information on both biological traits and population dynamics should be obtained for a better understanding of the actual consequences of removal of sunfish, from a whole ecosystem, in the context of anthropogenic pressure. Lastly, information such as that presented is relevant to assessing how climate change may be shifting marine predator spatial distributions and hence potentially altering their vulnerability to fisheries.

Chapter 7: General discussion

The work presented in this thesis results from the tracking the movements and behavioural patterns of sunfish, using three different satellite transmitters (Argos smart positioning only – SPOT, Argos linked Fastloc-GPS™ and pop-up satellite archival tags PSAT, Figure 35). Tracks obtained were generally of short duration (60 d on average) and possible causes for such extents should be explored. Thus, it seems appropriate to start this General Discussion with a brief comparison of the performance of the tags used (7.1). The main results from each chapter are then drawn together to contribute to a broader understanding of the behavioural ecology of sunfish (7.2). Main conclusions are then provided together with future work suggestions (7.3), including a putative ocean sunfish distribution map in the North Atlantic. Finally, a compilation of this study caveats is also provided (7.4).

Figure 36 Attachment of the three different satellite transmitters used to track sunfish movements: A) PSAT, B) Argos-SPOT and C) Fastloc-GPS™.

7.1 Satellite tag performance

Sunfish were tagged with Argos-SPOT tags at the tip of the dorsal fin (Figure 36B) which likely increased the chance for a location transmission via Argos whenever the fish was at the surface with the fin breaking the water surface so the tag was in air. The absence of a tether (employed in both PSAT and Fastloc-GPS™ tags Figure 36A and C, respectively), reduced drag and likely increased transmission chances. However, if weighted by the number of days at liberty, Fastloc-GPS™ tags outperformed Argos-SPOT tags in number of daily Argos locations. Towed Fastloc-GPS™ tags are expected to have greater stability due to the hydrodynamic housing (cone- or boat-shaped) when compared to both towed PSAT and fin mounted Argos-SPOT tags. Thus, tag shape is anticipated to have contributed to these performances. Hence, likely due to constant sunfish fin movements during swimming, SPOT tags are likely to have a reduced satellite acquisition, which confirms previous results (Hazel 2009, Costa, Robinson et al. 2010, Patterson, McConnell et al. 2010, Witt, Åkesson et al. 2010, Dujon, Lindstrom et al. 2014). Overall, even though we obtained a few long-tracks (> 90 d), battery drainage, salt-water switch and antenna failures, animal mortality and premature detachment may explain the majority of shorter tracks.

In total, three PSAT tags failed to transmit, probably due to transmitter hard-ware problems, which is frequently encountered in similar studies (Hays, Bradshaw et al. 2007, Hammerschlag, Gallagher et al. 2011). PSAT tags were programmed to detach from the fish after a pre-determined time (either 90 or 120 d) and no evidence for premature detachments were obtained. For six tags, the death of the sunfish was confirmed (>25d post-tagging), with free-floating tags transmitting the data and reporting a continuous depth for several days prior to release; such depth traces are consistent with sunfish sinking to the seafloor and remaining there prior to tag detachment. Lastly, only two tagged animals provided complete datasets for the entire deployment duration. Hence, similar to other PSAT studies, it seems probable that fish mortality caused the observed short term deployments in some cases in addition to tag failures (Graves and Horodysky 2008, Musyl, Domeier et al. 2011) which further supports the lower probability of failures associated with shorter deployments (< 180 d) (Hays, Bradshaw et al. 2007).

Signal loss in Argos-linked tags was not as easily explained and several hypotheses may be explored (see review in Hays, Bradshaw et al. 2007). Given the maximum track lengths obtained and the transmission rates per day being limited, battery consumption may be excluded as the sudden cause for transmission cessation. Similarly, premature release can also be discounted due to the attachment of these tags using stainless steel bolts to the tip of the sunfish dorsal fin. Thus, salt-water switch or antenna failure seem more likely for fin-mounted Argos tags. However, we obtained no evidence in the Argos diagnostic data to support the salt-water switch failure hypothesis. Notwithstanding, the dorsal fin placement of the Argos-SPOT with continuous acceleration and deceleration associated with lateral fin movement is likely to induce increased stress to the tag, especially the antenna (Holdsworth, Sippel et al. 2008). Importantly, the dorsoventral symmetrical morphology of the species (dorsal and anal fins) is responsible for a lift-based acceleration thrust in sunfish (Watanabe and Sato 2008). Furthermore, for the single Argos-SPOT for which no transmissions were received, it seems likely that it was a tag failure (Hays, Bradshaw et al. 2007, Hammerschlag, Gallagher et al. 2011). Lastly, none of the Fastloc-GPS™ tags failed to transmit and although providing the longer deployments (92 and 172 d) track length was generally short (< 45 d). In our study, the cessation of GPS-linked transmissions could not be attributed to fish mortality, as we would expect an initial period with no transmissions followed by constant uplinks as the tag re-surfaced after releasing from the sunfish carcass (due to scavenger action) (Sims, Queiroz et al. 2009). On the contrary, premature detachment was evident in some GPS tracks, with constant hourly Argos locations during 5 – 8 d, thus matching the frequency of a complete satellite revolution around the Earth (CLS 2011). This suggested that the tag released early from the fish and floated to the surface, uploading locations until the battery was exhausted.

7.2 Significance of findings

7.2.1 Foraging movements and behaviour

In Chapter 3 we investigated the environmental integration of the seasonal changes in the sunfish distribution in the north-east Atlantic. Our results, besides supporting the importance of water temperature as a driver (Sims, Queiroz et al. 2009), sunfish distribution was also associated with productive thermal fronts and upwelling systems. These results further support previous observations (Sims and Southall 2002, Sims et al. 2009). Importantly, the shelf seasonal movements of sunfish appear to be timely correlated with the productivity and thermal cycles in the region: a) summer northerly increased temperatures with intense upwelling at western Iberia and sunfish moving parallel to the Iberian coast towards northern latitudes; b) autumn decreased upwelling and cooling temperatures together with southwards movements of sunfish; d) cooler waters in winter and no upwelling coincident with southern latitudes occupancy by the fish, either at north-west Africa or into the Mediterranean waters; and e) warming of water temperatures at northern latitudes coupled with increased plankton productivity in spring trigger sunfish northwards migration. These north-south movements were associated with the upwelling frontal system boundary, similar to the sunfish displacements in the north-east Pacific Ocean (Thys, Ryan et al. 2015). Similarly, foraging was suggested to be driving the observed behavioural association as salps, a known gelatinous planktonic prey of sunfish (Nakamura, Goto et al. 2015), were mainly encountered in the vicinity of such upwelling fronts. Hence, not surprisingly, sunfish likely take advantage of increased foraging opportunities by remaining within highly productive areas, as fronts are known productivity hotspots (e.g. Scales, Miller et al. 2014).

Together with the horizontal tracking of movements, PSAT-tracked sunfish showed different depth profiles (nDVM, rDVM, surface oriented and irregular). All four of the different depth-use patterns were found over short periods of time, likely reflecting adaptations to prey vertical movements. However, given the necessarily lower accuracy trajectories reconstructed from PSAT-derived light level data, close linkage between sunfish vertical movements and environmental features or the water column stratification is still lacking. Interestingly, the vertical movements of sunfish tracked in this study were not restricted to daylight hours, as found for the sunfish off Japan (Nakamura et al. 2015). This major difference in diel vertical activity may be indicative of a feeding preference by sunfish in the north-east Atlantic for a wider range of prey, or prey with more varied behaviour at depth. It was with this lack of general information about the interactions between sunfish and their prey that the role of likely prey aggregation areas in the habitats selected by the sunfish were investigated with simulated monthly prey-fields (informed by the advection factor of oceanographic currents - Chapter 5). A principal motivation for this study was the observation that oceanographic currents have been found to account for the interannual abundance of gelatinous species in the North Sea (Lynam, Attrill et al. 2009). Moreover, for other zooplanktonic species (not jellyfish) no evidence was found of an ability to swim counter to the horizontal flow (Fossette, Gleiss et al. 2015). Thus, plankton distribution is expected to be mainly driven by physical processes. In this study, a positive correlation was found between generated plankton densities (PD) and CPR-gridded zooplankton abundance, supporting the role of currents in the ocean productivity. However, sunfish behaviour did not always result in better performances compared with than random performances from random-walk simulations; it was evident that seasonal differences occurred. When in areas of greater available productivity indicated by prey-field simulations, real fish performed similarly to null, random tracks. Importantly, the higher simulated particle density (apparent productivity) occurred in summer months and was associated with intense seasonal upwelling in the region. On the contrary, when in poorer simulated conditions (decreased productivity associated with winter months), sunfish tracks resulted in higher than random encounters with simulated particles (prey). These observations support theoretical and empirical results that show specific search strategies are adopted by a predator to increase feeding success when prey is sparsely distributed (Sims, Southall et al. 2008). Hence, results at the horizontal scale associated with simulated prey fields seem to conform to the idea that sunfish were optimising movements for foraging across different environments. Clearly, a major deficiency facing studies of pelagic predator behaviour is the lack of *in situ* measurements of prey abundance (Sims, Witt et al. 2006). Although video recording of feeding events (Nakamura, Goto et al. 2015) will improve the understanding of predator prey interactions in the marine ecosystem, in the present study however, no *in situ* data was obtained for sunfish potential prey. This is an area ripe for future investigations now that video/data-logging tags are small enough for routine deployments on even juvenile sunfish.

To investigate potential foraging behaviour in greater detail, sunfish fine-scale (GPS) behaviours were linked to the simulated productivity maps. Sunfish area-restricted searching (ARS) was found to be directly associated with regions of simulated plankton aggregations at various temporal scales. In addition, frontal regions were also linked to increased ARS occurrence, confirming previous hypotheses of strong associations of this species with thermally contrasting regions for increased foraging opportunities (Chapter 3, Sims and Southall 2002, Thys, Ryan et al. 2015). Moreover, at the base of the food chain, phytoplankton has been shown to be negatively correlated with SST, leading to increased primary consumer aggregations that propagate upwards to secondary consumers (zooplankton carnivores) (Richardson and Schoeman 2004). This study detected an optimum SST between 17º and 22ºC for sunfish ARS and a consequent decrease in this behaviour probability of occurrence with higher temperatures. Sunfish are likely to forage within this restricted thermal envelope coincident with high abundance of consumers that aggregate in higher phytoplankton concentration at lower temperature regions.

Importantly, sunfish dietary habits have been underestimated with the assumption that it is an obligate predator on gelatinous zooplankton (Bass, Dewar et al. 2005). Recently, a broader diet for sunfish relied on stable isotope analysis to infer prey based on trophic level (Syvaranta, Harrod et al. 2012, Nakamura and Sato 2014, Harrod, Syvaranta et al., 2013). These studies were very informative, but the lack of empirically identification of consumed prey led this study to carry out the barcoding approach to determine the sunfish diet composition (Chapter 4). A total of 41 different items belonging to five phyla were found, with sunfish feeding mainly on crustaceans and teleost fish and with cnidarian (the long-suggested predominant prey) comprising only 16% of consumed items. Therefore, although still mainly composed by pelagic species, this more generalist diet suggests preferences to be driven by the prey availability throughout the broad water-column occupancy of sunfish (Chapter 3), while also conforming to the apparent fidelity to higher (simulated) productivity regions (Chapter 5). Altogether, results from Chapters 3, 4 and 5 reveal an opportunistic feeding behaviour in sunfish, potentially following either steep gradients in temperature or areas of current-driven accumulation of potential prey.

7.2.2 Ontogenetic shifts

Although no adult sunfish (>1.4m) were tracked in this study and hence, no inferences on the wider population structuring can be empirically supported, results from Chapter 3 support an ontogenetic shift in occupied habitats. From the tracking movements, whereas larger individuals displaced greater distances, smaller tracked fish had a tendency to remain within the Gulf of Cadiz (GoC). This rather suggests that regional philopatry may occur in sunfish inhabiting the southern Iberia region, with smaller fish displaying increased site fidelity to the area. Additionally, and contrary to larger sunfish, smaller individuals tended to move with the direction of the major currents. Therefore, the increased daily displacements together with a positive *rheotaxis* observed in larger individuals may well reflect a greater ability to select a wider range of habitats, perhaps increasing the opportunities for foraging. These results are in line with a recent biologging study off Japan, where the behaviour of larger sunfish (> 1.00 m) was video recorded and showed they were feeding mainly on siphonophores at depths of 50–200 m (Nakamura, Goto et al. 2015). Although sunfish depth datasets in this study comprise only summaries of every four (or six) hours, a high degree of activity in tracked individuals in the north-east Atlantic was observed throughout the day and not confined to daylight hours as off Japan (Nakamura, Goto et al. 2015). The difference in size between sunfish tracked in this study [from 0.60 up to 1.00 m] and those by Nakamura et al. (2015), may explain the behavioural differences. We also detected a size-related depth occupancy, with smaller sunfish spending less time in deeper layers compared to larger individuals, which also displayed an extended vertical range. Hence, similar to the horizontal occupancy, vertical profiles of sunfish varied with the size of individuals and were probably related to diet preferences. Taken together, both diving behaviour and horizontal displacements suggest an ontogenetic shift in the space-use of this species, at least in the north-east Atlantic. Ontogenetic shifts in habitat use are widespread in mobile marine species, being found across diverse taxa from elasmobranchs (Grubbs 2010, Afonso and Hazin 2015) to teleosts (Macpherson 1998, Jones, Laurila et al. 2003). Different factors may drive this variation in space use, such as habitat availability, either in its resources or inter-specific dynamics, such as predator risk and competitive interactions. Overall, diet-related processes are expected to either constrain or expand a species' geographic range, and which may could also account for the current results for sunfish.

In support of an ontogenetic-related habitat selection hypothesis, analysis in Chapter 5 revealed that whereas larger individuals appeared to feed solely on hydrozoans, scyphozoans and malacostracans, smaller fish diet included teleosts, hydrozoans, malacostracans, bivalves, gastropods and cephalopod preys. Thus, although no adult fish were sampled (>1.4m), a clear size-related diet was reported, conforming to the recent suggestion that this fish switches from a more benthic predatory pattern of behaviour to greater exploitation of the water column with growth (Syvaranta, Harrod et al. 2012, Harrod, Syvaranta et al. 2013, Nakamura and Sato 2014). The finding of a variety of prey items in smaller sunfish sampled in the same geographic region and at the same yearly season over two consecutive years, suggested the productive region of the Gulf of Cadiz provides abundant and diverse feeding opportunities for the species. In fact, this region has been described as an important nursery area for other teleosts with constant recruitment to the area (Bellido, Pierce et al. 2000, Catalán, Jiménez et al. 2006). This is of more importance when linked to the reduced displacement found for smaller sunfish probably linked to the available productivity, and for which diet was found to be mainly composed of coastal and/or benthic prey. Larger sunfish on the contrary dispersed widely, probably mimicking the seasonal latitudinal abundance changes in zooplankton in the North Atlantic, and showed an increased ability to swim counter-advected major currents. This assumption conforms to the finding of exclusively pelagic prey DNA in larger sunfish stomach contents. Moreover, the rare occurrence of mature individuals in the set-net (tagging region) also suggests a more oceanic habitat preference for larger individuals. Altogether, it is suggested that the variation in the dietary habits underpin sunfish size-related geographical occupancy. The satellite tracking of movements and diving behaviour of larger individuals (above the maturation size) coupled with dietary preferences, would greatly contribute to a more complete picture regarding the ecology of this species. Further studies should now focus on whether sunfish movements are driven by the species dietary habits or if the diet changes in response to size-related shifts in occupied habitats.

7.2.3 Conservation

The size segregation we observed in north-east Atlantic sunfish, both in spatial use and feeding dynamics, may have further implications for conservation and management. The understanding of the mechanistic processes underpinning size-related shifts is crucial for managing the risk posed by fisheries to either target or bycatch species (Snover 2008). Despite the high level of recorded bycatch in different fishing activities (Silvani, Gazo et al. 1999, Cartamil and Lowe 2004, Petersen

2005), the low commercial value of sunfish (since it is only consumed in few places in the world) has driven the lack of accurate estimates of exploitation levels of this species (Pope, Hays et al. 2010). In the European Union, sunfish and other Molidae species are banned from commercialisation, following the Regulation (EC) No 853/2004 of the European Parliament[9]. However, reported bycatch from different regions is still surprisingly high given the lack of commercial interest. For instance in the western and central Pacific Ocean shallow-set pelagic longline fisheries the sunfish is the most captured teleost other than the targeted species (Clarke, Sato et al. 2014); whereas in the western Mediterranean mesopelagic longline fishery a sharp increase in sunfish catches was recorded, despite the documented release at sea (Garibaldi 2015).

In Chapter 6 we present a first attempt to estimate the susceptibility of sunfish to longlining fishing operations in the north-east Atlantic. Results show a positive correlation between tracked sunfish space-use and areas of intense activity by two major longlining fleets (Portuguese and Spanish). Longlining fisheries frequently capture sunfish on the baited hooks, either by actively feeding on the bait or by being accidentally captured (foul-hooked in the body - Figure 37A), with fish being later released but with no empirical information likely survival rates.

Our results show a seasonal pattern in both fish and vessels concomitant space use, likely due to the seasonal distribution patterns of the species. Moreover, the overall estimated number of days at risk per month was similar to the rate found for blue sharks over the entire North Atlantic basin (Queiroz, Humphries et al. 2016). However, this shark species is a targeted resource and one of the most frequently captured species by longlining fleets (Camhi, Lauck et al. 2008). Yet, the undesired and non-commercially valuable sunfish presented an equivalent risk to the species and burden to the fishery. Importantly, sunfish vulnerability to fishing fleets was significantly higher when compared to random (null) tracks. Hence, there is a need for reporting of detailed captures

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[9] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R1020&rid=2

since, at least for the north-east Atlantic, we showed that the long lack of compulsory monitoring have hindered the assessment of susceptibility of sunfish to longlining activities. Furthermore, a multitude of fisheries, with very high incidences of sunfish catches, have yet to be analysed (Silvani, Gazo et al. 1999, Cartamil and Lowe 2004, Tudela, Kai Kai et al. 2005, Thys, Ryan et al. 2015). Assessments of captured sunfish in gillnetting, driftnets and trawling need to be made and should be further incorporated within future dedicated studies.

Changes in the population structure are expected in response to increased fishing efforts which will ultimately increase fish susceptibility to other threats, such as climate change (e.g. Planque, Fromentin et al. 2010). Analysis of population structure and realistic fishing risk assessments for sunfish should then follow the present study. Given the differences in regional occupancy between larger and smaller sunfish, from coastal while juveniles to deep offshore waters in adults, we might expect this species to be particularly susceptible to climate variability (Ong, Nicholas Rountrey et al. 2015). Climate change impacts are thought to differ in form, incidence and severity in different oceanic habitats; for example shallower coastal waters are expected to have an accelerated warming, varied salinity and water column stratification due to altered fresh water inputs due to changes in rainfall and terrestrial run-off patterns (Rabalais, Turner et al. 2009, Doney, Ruckelshaus et al. 2012). Hence, different regions and the species that inhabit them are likely to be susceptible to varied impacts in the current climate change scenario. If sunfish are indeed selecting different habitats with growth, different impacts may affect the different components of the population. Longer tag deployments on a wider range of sunfish body sizes are desirable in order to confirm the occurrence of a partial migration in sunfish in the north-east Atlantic.

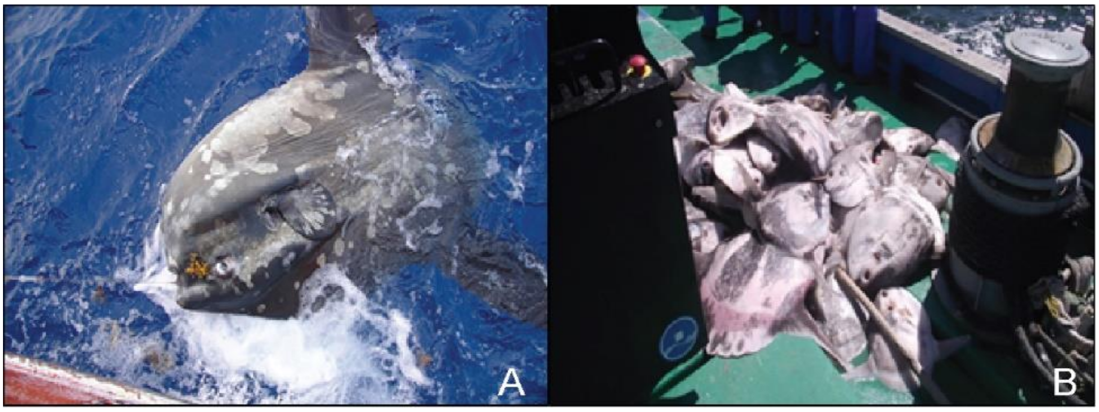


Figure 37 Pictures of captured sunfish in two different fishing gears A) longlining and B) set-net

7.2.3.1 Policy-makers influence

The results presented over the five experimental chapters are important outcomes that better informed the ecology and conservation of ocean sunfish, at the north-east Atlantic scale. The publication of these results as a series of scientific papers (which are now being prepared) will contribute to the general knowledge on this charismatic species' distribution and overall behavioural patterns.

The reported focal residency of sunfish in highly productive regions, with track-estimated habitat selection being driven by SST and highly influenced by thermal gradients (chapter 3 and published in Animal Biotelemetry 2016) further supports the ecological importance of frontal regions for mobile marine vertebrates as potential priority conservation areas (see Scales, Miller et al. 2014). The habitat model designed in Chapter 3, based on telemetry data, revealed the interactions of sunfish relative to environmental features, which can serve as a tool to guide conservation and management decisions. Importantly, the relationships quantified here predicted a distribution beyond our recorded observations, where none of our tracked sunfish travelled but where sunfish are known to aggregate, like the south-west peninsula of the UK which in previous studies has been shown to have high sunfish abundance in summer months (Leeney, Witt et al., 2011, Sims, Southall et al., 2002). Thus, we were able to provide a more realistic and mechanistic interpretation of system dynamics than static approaches. Marine biodiversity conservation will likely benefit from a marine spatial planning, targeting known biodiversity hotspots, and within Exclusive Economic Zones and in the open oceans. Ultimately, high productivity regions may be ideal habitat targets for the implementation of high seas marine protected areas (MPAs) for a number of large pelagic species, from jellyfish (Graham, Pagès et al. 2001), turtles (Fossette, Hobson et al. 2010, Polovina, Kobayashi et al. 2000), marine mammals (Dragon, Monestiez et al. 2010) and teleost fishes (Lutcavage, Brill et al. 2000, Walli, Teo et al. 2009, Seki, Polovina et al. 2002), among others.

Longlining is known to target persistent frontal zones (Podestá, Browder & Hoey 1993, Hartog et al. 2011), particularly within the coastal zone, along shelf breaks and in upwelling regions (Halpern et al. 2008; Lewison et al. 2014). The high-space use displayed by ocean sunfish, focused mainly in such coastal fishing areas crossing national political-economic boundaries, further complicates any future management or conservation initiatives. Importantly, in these regions mitigation measures should consider the effects across the range of potential bycatch and target taxa. In fact, the effective implementation of management measures, that can protect bycatch populations as a whole, is likely to be the best approach in the region.

One of the greatest ecological threats facing the oceans is overfishing and its associated bycatch, yet catches of many species remain largely unregulated with poor data reporting. One such case is the ocean sunfish which has recently seen its status re-evaluated to Vulnerable by the IUCN Red List of Threatened Species committee, given the high risk of extinction as a consequence of the increased levels of captures reported worldwide. For sunfish the absence of adequate reporting of captured individuals in different fishing techniques has hampered a correct estimation of the effects such anthropogenic activities exert on the populations worldwide. The high overlap between sunfish and longliners operating in the north-east Atlantic presented here urges the implementation of a better reporting which ultimately may inform effective mitigation effects measures. The absence or the inconsistency in the data reported hampers the estimation of a correct number of sunfish bycatch rates, either between fisheries or over time. Hence, to ensure transparency, standard methods for collecting and reporting bycatch are vital to assist future assessment and mitigation efforts on the catch of non-target species. Importantly, post-release mortality is yet unquantified and thus, there is still a lack in our understanding on the actual impacts longline fishery pose to sunfish. Lastly, other fisheries such as drift- and gillnets also affect sunfish worldwide and the same strategy of a more efficient data collection and assessment programmes is likely to provide scientifically robust data to holistically address the status of this species' actual bycatch. Reported bycatch of sea turtles for instance, instigated the use of circle hooks proven to be a viable option for the mitigation of the species' unintentional catch in the Brazilian pelagic longline fishery (Sales, Giffoni et al., 2010). In fact, in a recent review gear modifications consistently reduced bycatch of leatherbacks Dermochelys coriacea, black-footed albatross Phoebastria nigripes and showed strong promise for vaquita porpoise Phocoena sinus (Senko, White et al., 2014). Yet, although many gear modifications reduce bycatch in experimental trials, the actual practice in commercial fisheries is less effective (e.g. Campbell & Cornwell, 2008). Importantly, this lack of effectiveness in applying such mitigation measures in fishing fleets may have serious consequences for management and conservation of protected species taken as bycatch (Cox, Lewison et al., 2007).

7.3 Future work

Habitat selection, behavioural patterns, diet identification, predator-prey modelling and susceptibility to fisheries of the ocean sunfish have all been considered in the present study. The current investigation is probably the most complete single study on the behavioural ecology of sunfish yet attempted. As a result, important species specific dynamic traits including spatial behaviour in relation to the immediate environment and ecological interactions with other taxa were revealed.

7.3.1 Fine-scale behaviour patterns recording

In this study, sunfish behaviours are still estimated from track metrics and finer-scale recorders could help in our understanding of such specific patterns. One example are the electronic data storage tags (DST), which have the impressive capacity to store high resolution data to non-volatile flash memory for as much as 20 years (Sims, Wearmouth et al. 2006). This storage capacity enables both temperature and depth to be recorded in a *near-continuously* way. As an example, preliminary analysis on the fine-scale data of depth occupancy (at every 1 or 10 seconds) of sunfish tagged with DST inside a semi-closed set net off southern Portugal enabled the description of sunfish fine-scale depth occupancy and for some hypothesis to be tested. Specifically, preliminary results revealed fish step-lengths' are better defined by a Lévy-walk-like movement for as much as 64% of the time. Levy walks are theoretically optimal search patterns where prey is sparse and patchily distributed and foragers have incomplete information about where resources are located. This preliminary result is consistent with the Lévy flight foraging theory in which animals are not obligatory Lévy foragers for all the times. In fact Lévy-type movements are expected to maximise foraging efficiency by enhancing the encounters of sparsely distributed high density fields of zooplankton (Humphries, Queiroz et al. 2010). Interestingly, a diel shift from Lévy to Brownian-type movements was detected in several individuals, and both scenarios (day – Lévy or Brownian and night *vice versa*) were observed. Figure 37 gives an example of a sunfish depth profile and respective diel MLE distribution patterns. Thus, empirical evidence on the occurrence of this behavioural pattern further confirm north-eastern Atlantic sunfish to be active both day and night, likely reflecting different foraging opportunities. This result is in line with recent video-recordings of sunfish in the Mediterranean, where daily activity was also found (Phillips, Harrod et al. 2015). Another example are the accelerometer data loggers (LAT1810ST; Lotek Wireless Inc., Newmarket, ON, Canada) coupled with an animal-borne camera with a light source in sunfish off Japan revealed decreased fish body temperatures during deep excursions and afterwards recovered during surfacing periods (Nakamura, Goto et al. 2015). Timing of feeding together with the type of ingested food were recorded for the first time for sunfish and it seems a logical follow-up procedure to our study in the region. However, smaller individuals should also be monitored given the size-related shift in the diet preferences in this species. In fact, biologging provides crucial insights into otherwise largely inaccessible biological systems (Rutz and Hays 2009). The disadvantage of requiring the animal to be re-captured for tag/sensors recovery is no longer a problem as new packages with a retrieval system consisting of a time-release mechanism are now available (see Houghton, Liebsch et al. 2009, Nakamura, Goto et al. 2015). Although further experiments are needed, future analysis on the sunfish behaviour at the finest scale are promising for more complex questions to be answered.

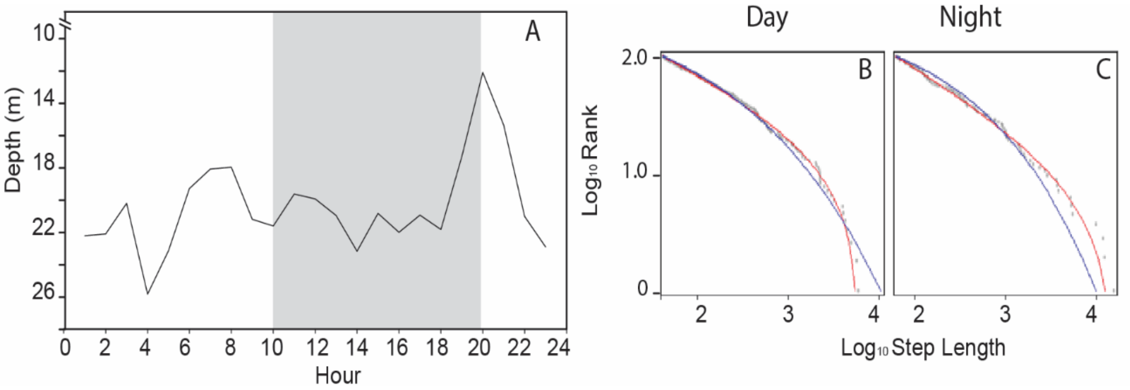


Figure 38 Example of a sunfish depth profile recorded with a data storage tag (A). Diel MLE distribution patterns of sunfish step lengths are given in B (day – TP Lévy type movement) and C (night – Exponential Brownian type movement).

7.3.2 Population dynamics

Another missing aspect of sunfish biology is the reproduction and recruitment assessments of this species in the Atlantic. In a study off Japan, sunfish spawning period was found to be relatively long, from late August to late September, being confirmed that this species is a multiple spawner (Nakatsubo, Kawachi et al. 2008). No similar studies have been made elsewhere and the reproductive biology of sunfish in the Atlantic remains unknown. Hence, despite not being a focus of this thesis and for which the tracking of mature individuals would have greatly contributed for the determination of spawning sites, our results along with data from other sources as the oceansunfish.org and previous tracking studies, can be brought together to provide a putative ecology for North Atlantic sunfish (see Figure 39). Given the observed displacement of larger individuals away from the Gulf of Cadiz we infer that this is not a spawning region for sunfish, rather this region emerges as an important nursery ground for smaller fish. Both Atlantic and Pacific sunfish tagging studies suggested a coastal residence of the species, at least for some size groups (Sims, Queiroz et al. 2009, Sims, Queiroz et al. 2009, Dewar, Thys et al. 2010, Potter, Galuardi et al. 2010, Thys, Ryan et al. 2015) where no evidence of *M. mola* movements across ocean basins was found. Our findings also confirm a coastal residency for sunfish following the regional annual cycles of productivity and without movements into the mid–North Atlantic. All these studies report the species engagement in latitudinal movements within 300 km from shore and correlated with either increased productivity or avoidance of extreme temperatures. Nonetheless, no evidence of very small sunfish or larval recruitment has been found in either region.

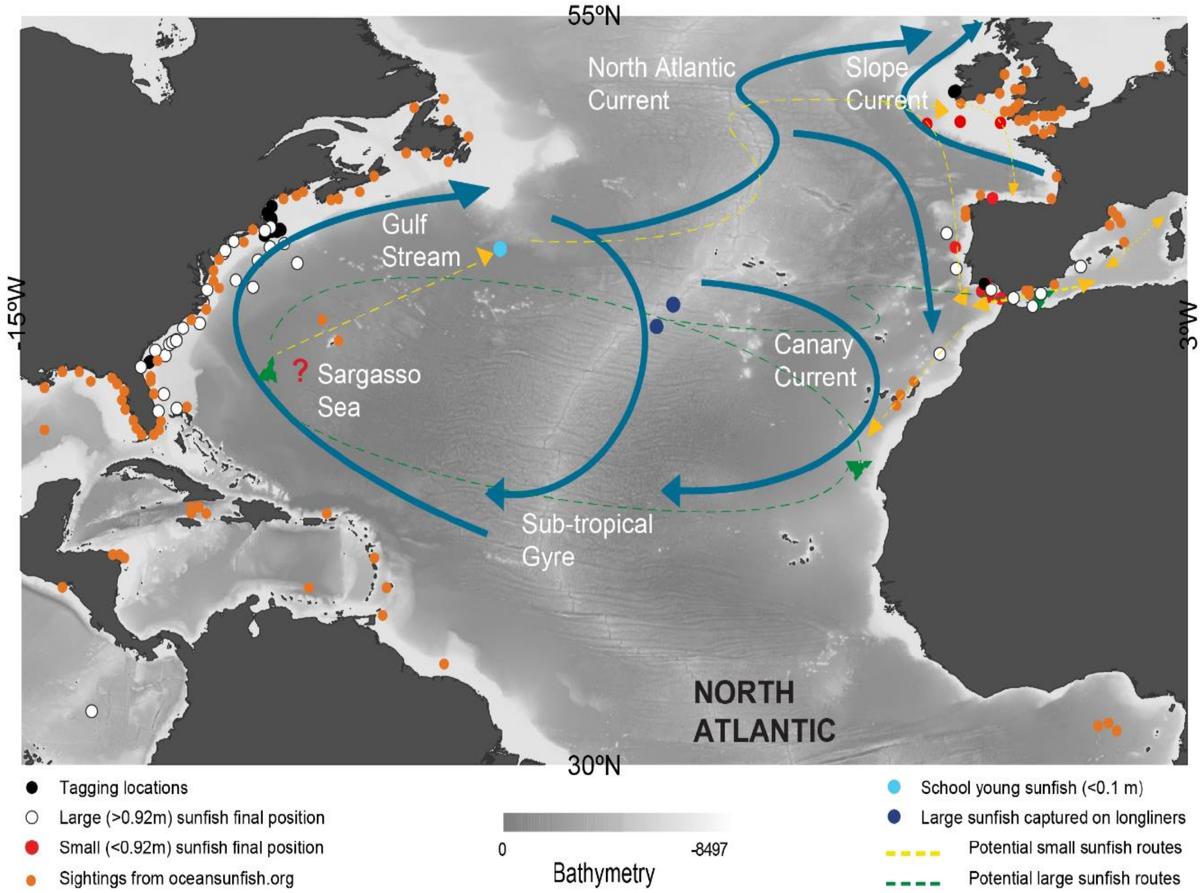


Figure 39 Map showing the recorded sunfish occurrence in the North Atlantic. Tagging data gathered from Potter et al., 2011, this study. Black dots: tagging locations; white and red dots: last positions (large and small sunfish respectively); orange dots: sightings from oceansunfish.org; light-blue points to small sunfish school observed from a longliner; dark-blue show large sunfish captured in longliners; yellow arrows: potential routes for small sunfish spawning in the Sargasso Sea (given by the red '?'); green: larger sunfish routes.

Notwithstanding, mature individuals are frequently caught in longliners operating in open waters in the North Atlantic (Mucientes G. *personal communication*, Figure 40A). These observations of larger individuals support the hypothesis that sunfish spawn in the Sargasso Sea or in the vicinity of open ocean seamounts, or perhaps both. Furthermore, in 2010 a school of thousands of very small sunfish (~ 0.10 m TL) were observed passing a fishing vessel by one of the research team for as much as three days at the position 40ºN 48ºW in the Gulf Stream (Figure 40B).

These observations rather link sunfish spawning to the Sargasso Sea, with post-larval stages being carried first north then east in the Gulf Stream, a similar pattern to the well-known European eel (Riemann, Alfredsson et al. 2010). Different teleost and shark species were already associated with this region (see Luckhurst 2014) with for instance, strong evidence for this area to be a pupping ground (the porbeagle shark Campana, Joyce et al. 2010). Although oligotrophic, the availability of dinoflagellates and ciliates in the area potentially leads to increased mesozooplankton secondary production, and subsequently augmented availability of energetic gains to organisms at higher trophic levels (Andersen, Nielsen et al. 2011). Sunfish diet encompasses a wide range of planktonic organisms, including gelatinous zooplankton and copepods (Chapter 5), a similar dietary preferences to that of larval eels feeding in the Sargasso Sea (Riemann, Alfredsson et al. 2010). Hence, with energetically profitable feeding opportunities for sunfish, the Sargasso Sea may be a suitable spawning region. In this case, sunfish larvae/juveniles would follow the Gulf Stream and North Atlantic Current towards northern latitudes appearing in the Celtic Sea (Houghton, Doyle et al. 2006); or the Azores Current towards the Canary Current and approaching the productive region of the Gulf of Cadiz (Chapter 3 and Sims, Queiroz et al. 2009, Sims, Queiroz et al. 2009) (Figure 39). Altogether, these assumptions are in line with the reported overlap of both *M. lanceolatus* and *R. laevis* spawning in the region (Bass, Dewar et al. 2005).

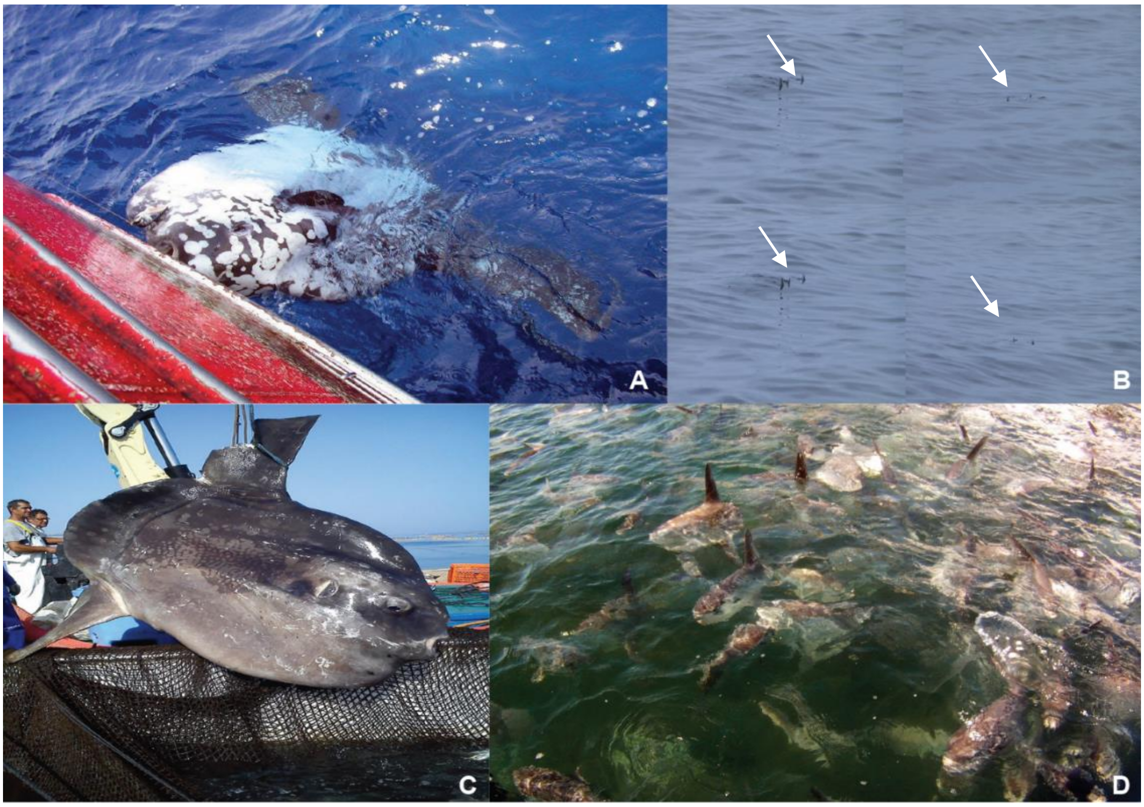


Figure 40 A) Image of a large sunfish captured on a longliner operating in the North Atlantic; B) frames recovered from a video recording of a school of sunfish (n > 100s) during a longlining observation program in the North Atlantic; C) Small sunfish > 0.20 m entrapped inside a set-net targeting tuna.

Larger individuals have also been only occasionally observed within the Mediterranean Sea, another largely oligotrophic area, and rarely have sunfish of such size entered the set-net (Figure 40C). Interestingly, the three largest tracked sunfish in this study entered the Mediterranean in 2013 (#15, #17 and #18). Although sizes did not exceed the maturation TL, these movements could reflect pre-adult migrations to Mediterranean spawning areas, similar for instance to those of the bluefin tuna (e.g. Druon, Fromentin et al. 2011, Aranda, Abascal et al. 2013). However, if the Mediterranean is the influx source of sunfish observed in southern Iberia, we would expect smaller sunfish of equivalent (or smaller) sizes to the ones observed near the Sargasso region, to be recorded there. This, to our knowledge has never occurred. Sunfish are the most fecund of all vertebrates (Pope, Hays et al. 2010) and even though the species has an impressive growth rate (Nakatsubo, Kawacxt et al. 2007), sizes of sunfish within the set-net operating in Southern Portugal over the years (> 0.20 m, Figure 40D) rather suggest a spawning area farther away. Determining the spawning timing and breeding or spawning areas of sunfish in the North Atlantic are clearly targets for further research.

7.3.3 Study limitations

Several aspects of this study were hampered and should be accounted for in future work.

a) The major drawback in this study is the current limitation imposed by tracking systems inaccuracy, with the interpretation of behavioural information being hampered by the missing links of specific traits, such as feeding events to the actual observed behaviours of sunfish;

b) The small sample size (18 successfully tracked individuals);

c) We need to acknowledge a possible bias in the interpretation of movements and space-use of our tracked individuals which were all released in the coastal zone (e.g. Nakano and Stevens 2008);

d) The interpretation of the diving patterns which was performed individually and according to each significant behavioural change, should have been analytical linked to the bathymetry as depth is likely influencing the water column structure. Not only the diving profiles should be sectioned by significant changes in the behaviour (as they were) but also, according to the different region specific bathymetry.

e) The majority of individuals found in the set-net field site we used are immature with total lengths rarely exceeding the maturation size (Nakatsubo, Kawacxt et al. 2007), hence inferences on population traits could only be suggested. The better understanding of sunfish in the north-east Atlantic will definitely benefit from tracking adult individuals, which likely occur in higher numbers oceanic regions (given the higher encounter rates with larger fish on oceanic longlines);

f) Little or no knowledge of age, sex, population status of the studied animals, further dedicated studies should focus on the population dynamics in the region, e.g. are populations off Iberia and Ireland connected?;

g) Investigation of secondary and/or tertiary predation should be taken into account and, for instance, the application of the same DNA barcoding framework to the larger species identified as prey of sunfish (Chapter 4) would likely confirm if any of the items were not directly consumed by the fish;

h) A barcode can only identify a species based on sequence similarity in a database; therefore, an unknown species can only be accurately identified if a representative species barcode exists in the database (e.g. GenBank). Despite the efforts to detect the maximum consumed prey items possible (e.g. all possible mitochondrial translations were performed – Chapter 4) the identification was dependent on the available sequences in the database, which for larger gelatinous may be less efficient;

i) In Chapter 5, in shelf environments, tidal-mixing fronts are likely to be as or more important than eddies to validate the simulated plankton fields. It was detected in the study that steep gradients in the temperature – a proxy for fronts in the region (e.g. upwelling, tidal-mixing) were driving sunfish ARS by likely exerting a greater control on plankton aggregation areas. Hence, in this specific case, thermal frontal features would probably be a better validation for the simulated fields;

j) The sampling of gelatinous zooplankton at near surface (<9 m depth) by the CPR used to validate the simulated prey aggregation areas (Chapter 5). These CPR surveys lack rigorous quantification of gelata, which is likely biased towards small species, individuals or body parts (Witt et al., 2007). Hence, CPR data is probably not the best solution to validate the simulated fields in Chapter 5. In fact, the foraging patterns we found and the link with simulated current-driven planktonic densities (Chapter 5) would greatly benefit from *in situ* measurements of for instance teleost larvae, coastal crustacean and pelagic cnidarian species (all found to be directly consumed by sunfish in Chapter 4);

k) No precise information on captured sunfish in longliners analysed (Chapter 6). Fisheries interactions with sunfish would greatly benefit from more precise abundance proxies, such as catch per unit effort (CPUE).

Appendix A Supplementary material

A.1 Chapter 3 : Supplementary results

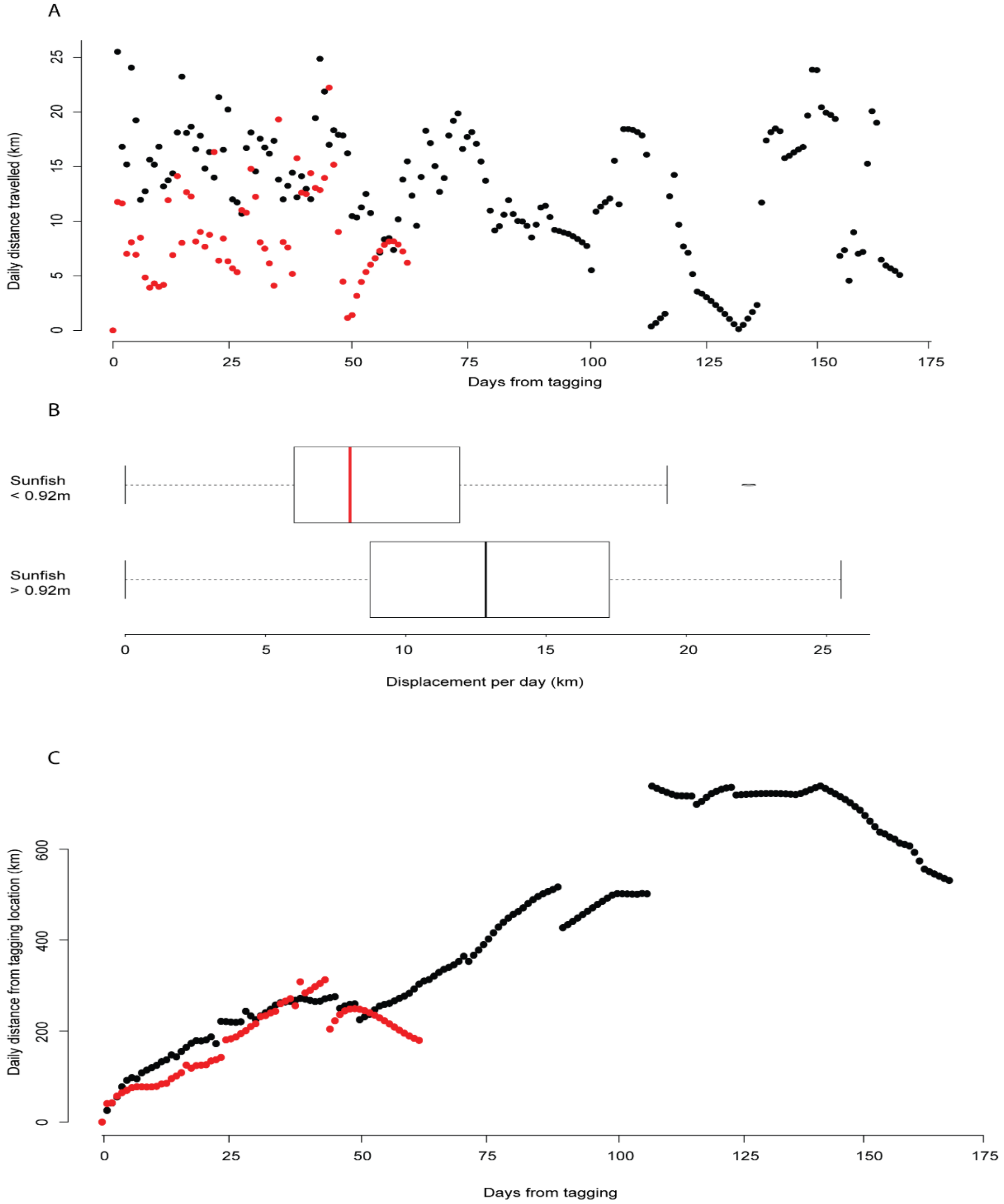


Figure S1 Sunfish displacement per size. A) Daily displacement in relation to time coloured according to sunfish size; B) Boxplots of the size related displacements; C) Average daily distance from tagging location, per Julian day coloured by sunfish size[Red – sunfish smaller than the average TL (0.92 m) and black for larger individuals].

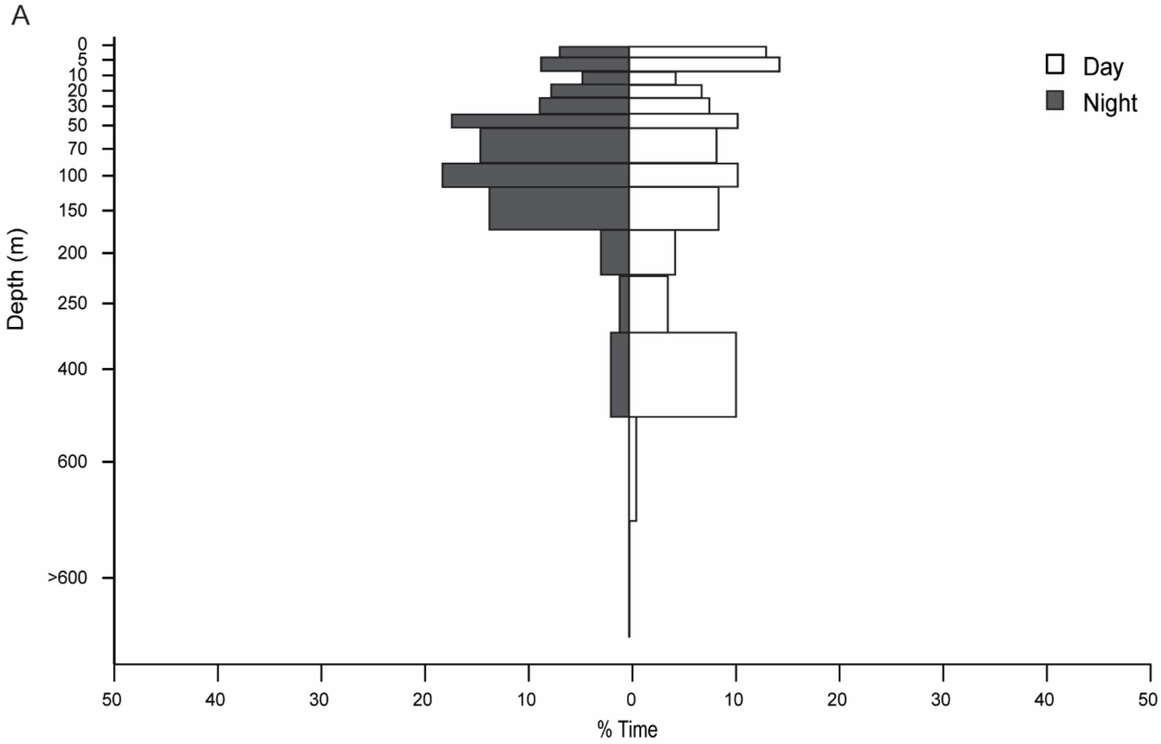
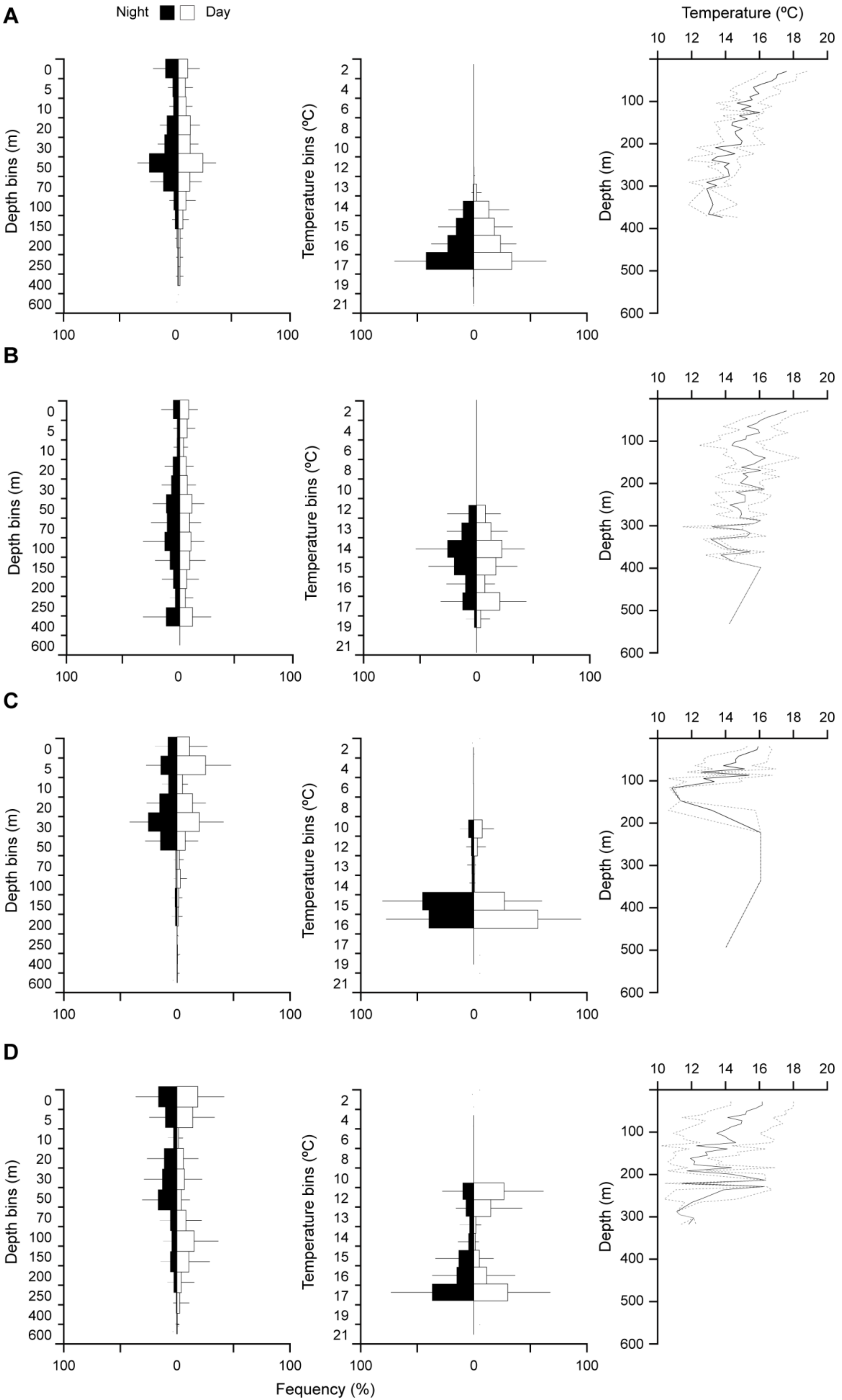


Figure S2 Sunfish time at depth histograms. General depth occupancy for tracked sunfish pooled from the entire dataset (*n*=18).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Temperature (ºC)
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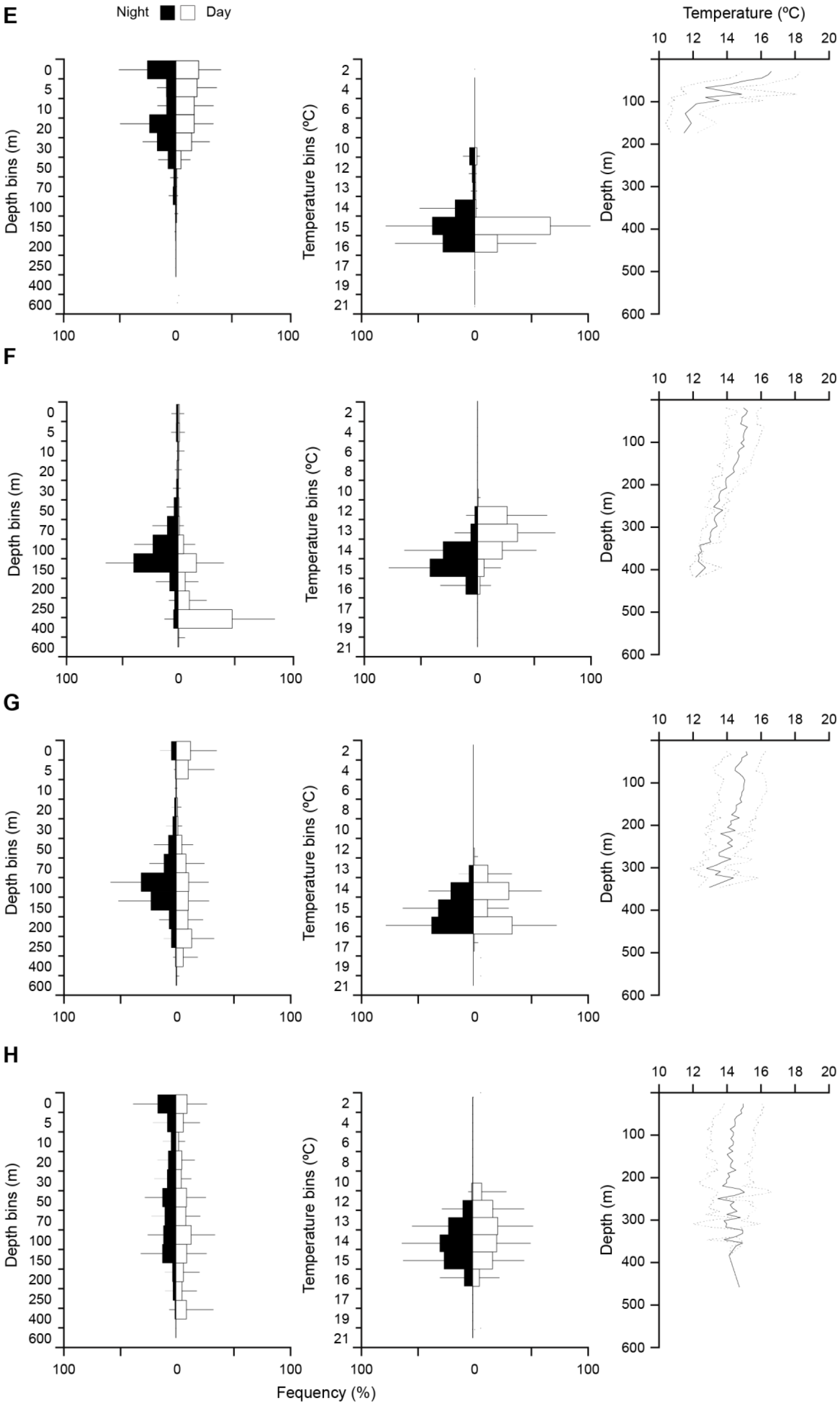
Fequency (%)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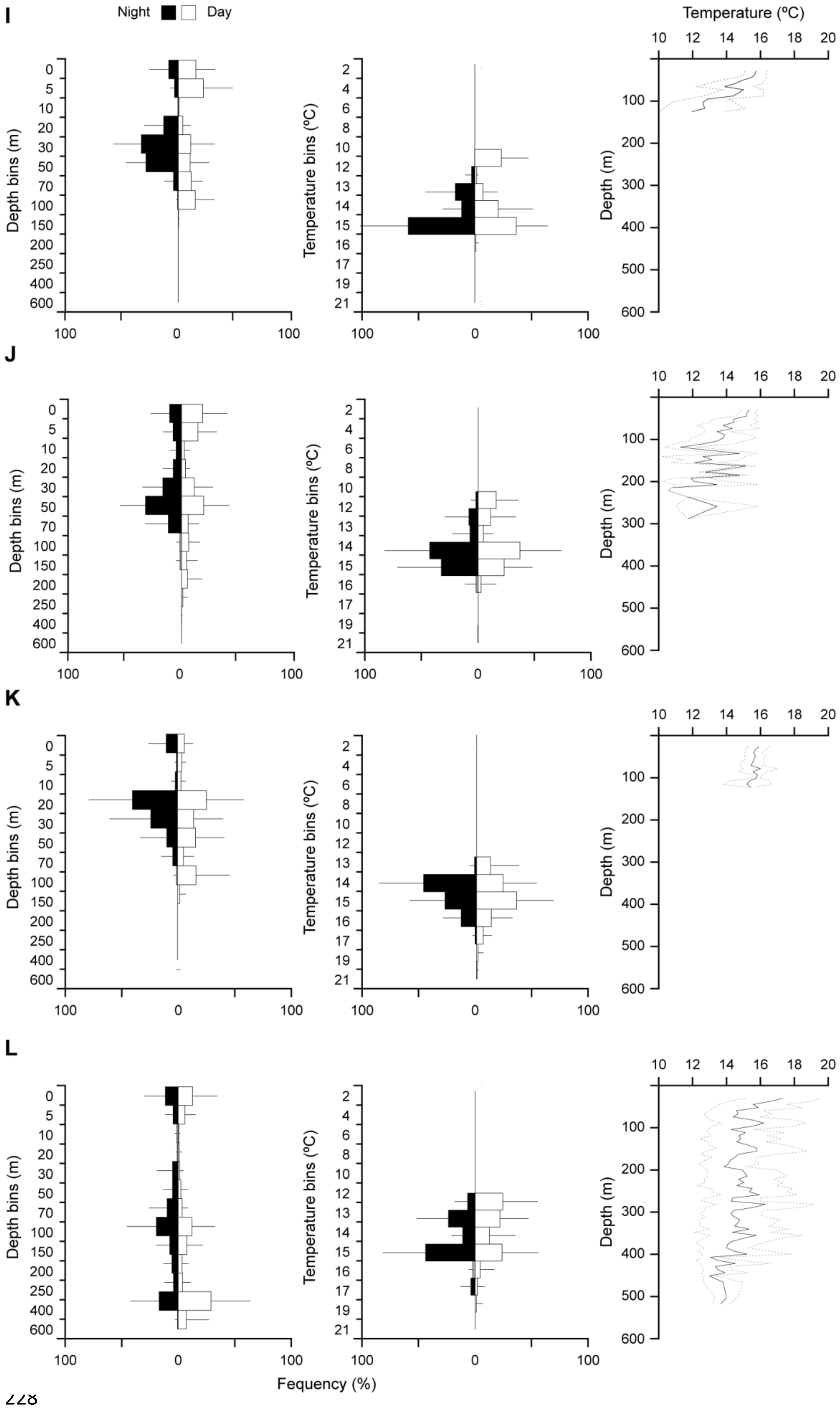
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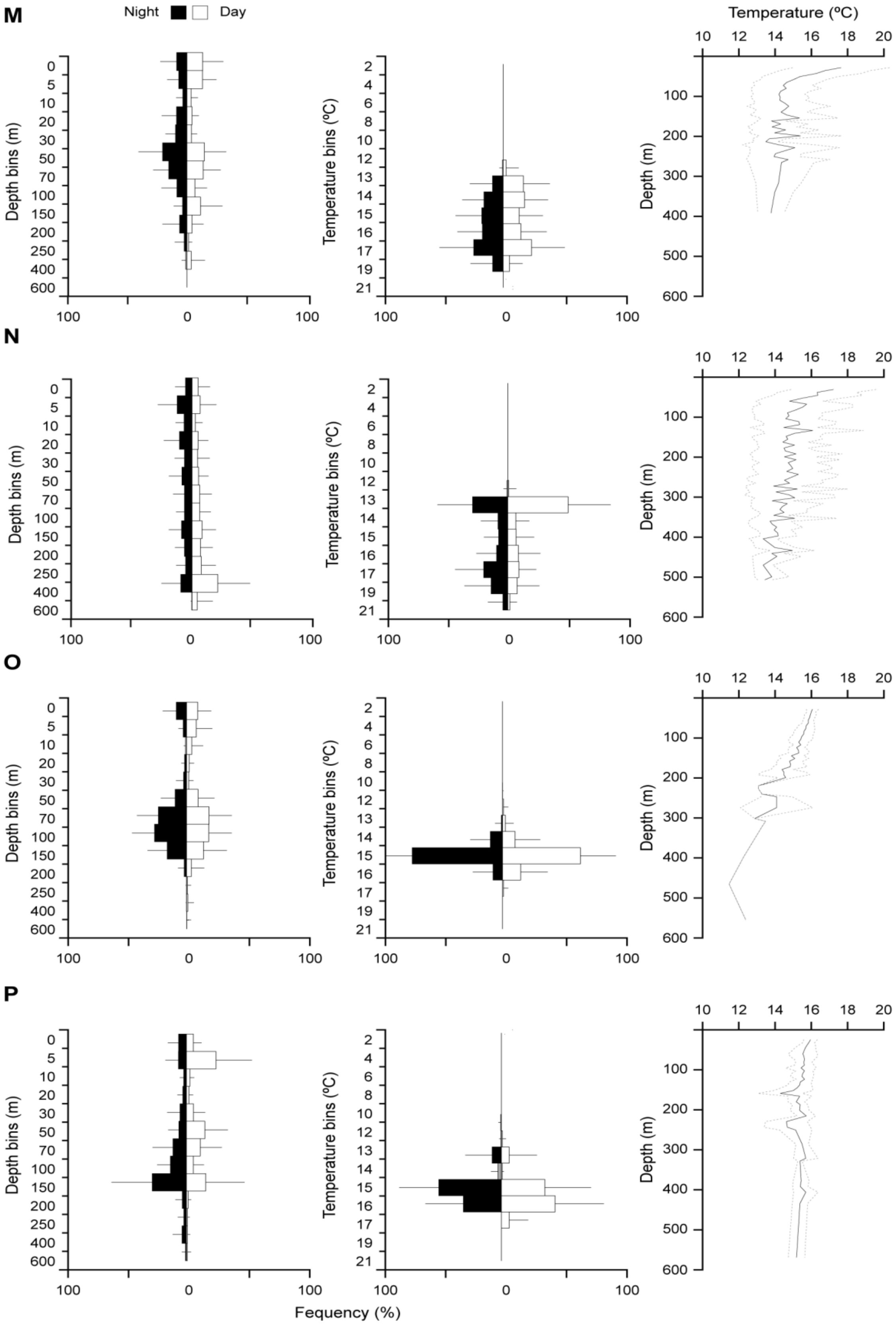

Figure S3 Individual diving profiles (TAD – left; TAT – central; water column structure – right panel) for each of the remaining 16 SMW sections defined in this study.

A.2 Chapter 4 : Supplementary results

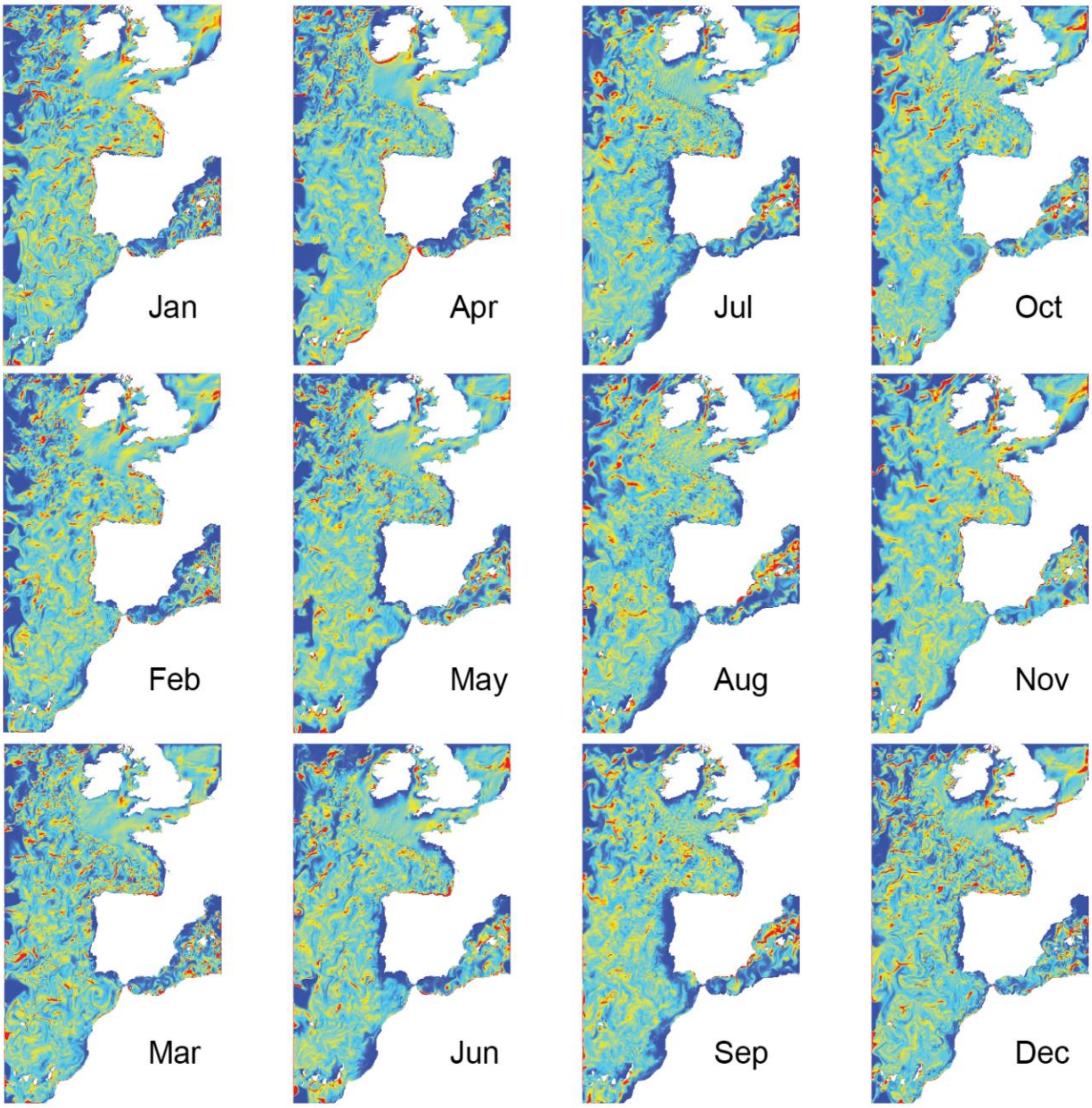

Figure S4 Monthly maps of generated plankton densities pooled from the period 2007-2011.

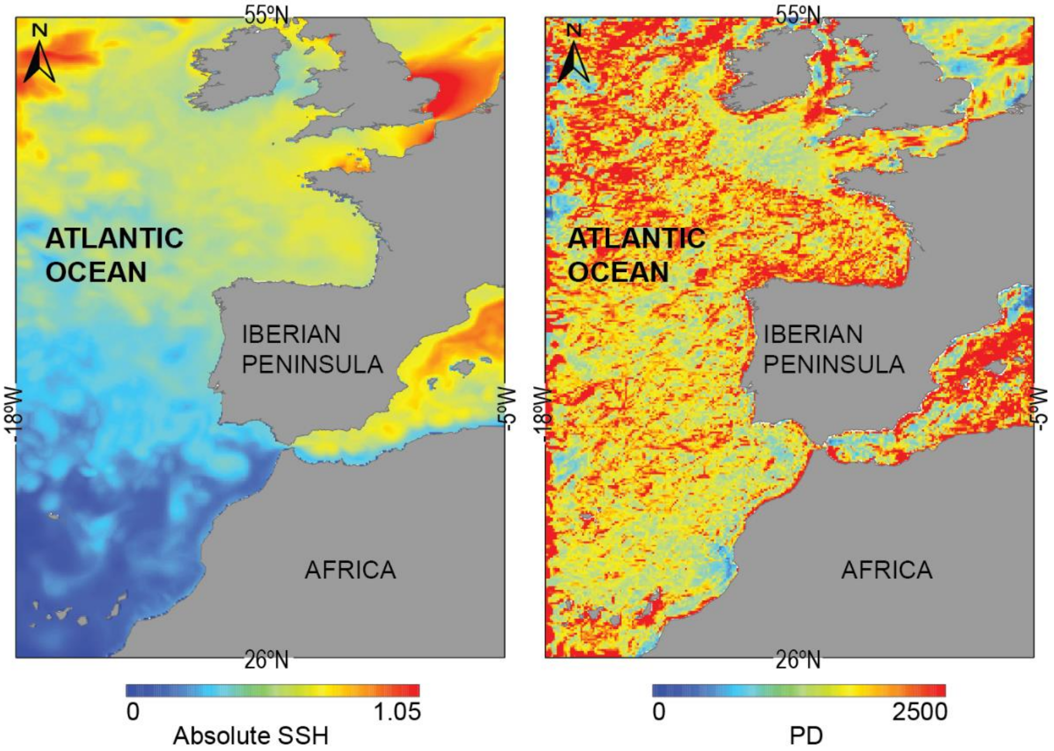

Figure S5 Maps of high resolution absolute maxima of SSH A) for the tracking period (2007-2013) and B) maximum PD index generated, for the same period.

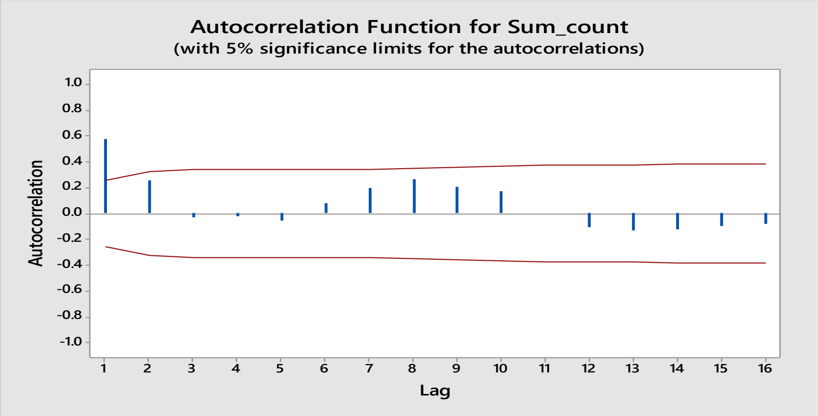

Figure S6 Plot of autocorrelation of sunfish time spent per grid cell of generated PD index.

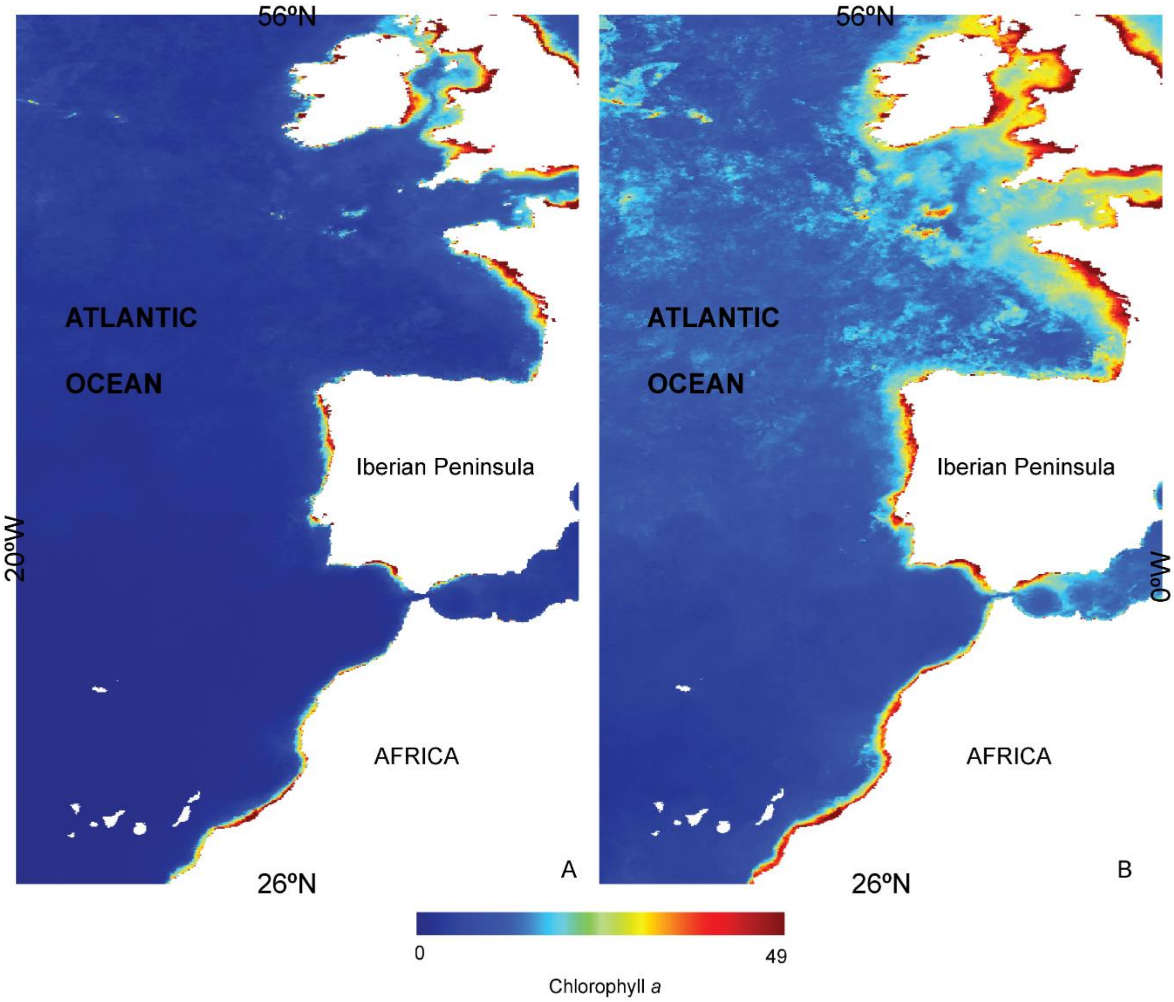

Figure S7 Productivity in the region measured by chlorophyll *a* overall maps for both winter (A) and summer (B), pooled for the entire tracking period (2007-2013).

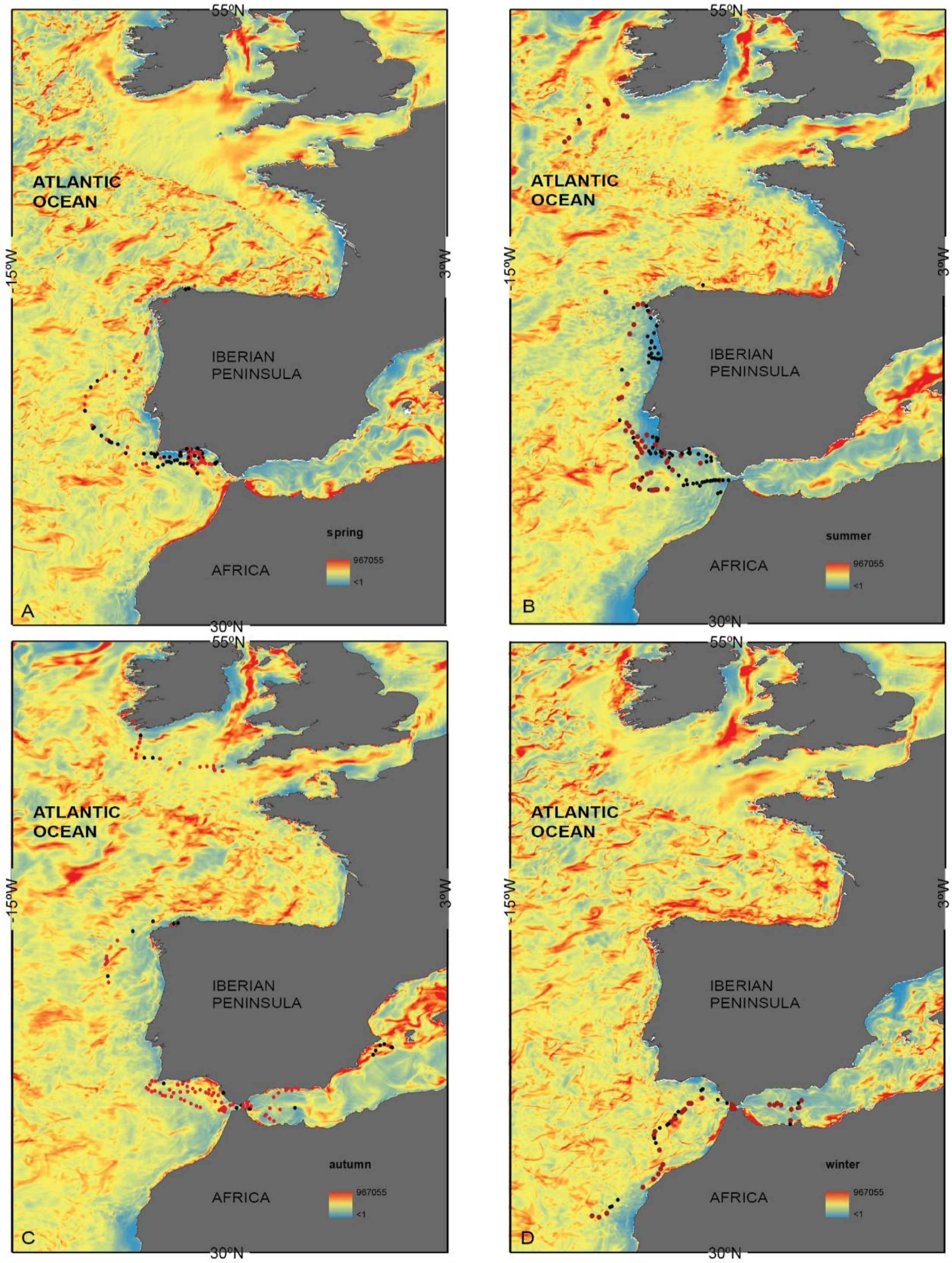

Figure S8 Map showing all sunfish tracked positions coloured in red if real PD higher than the random (null tracks), and coloured black otherwise. Background colours show simulated plankton density (warmer colours indicating higher densities).

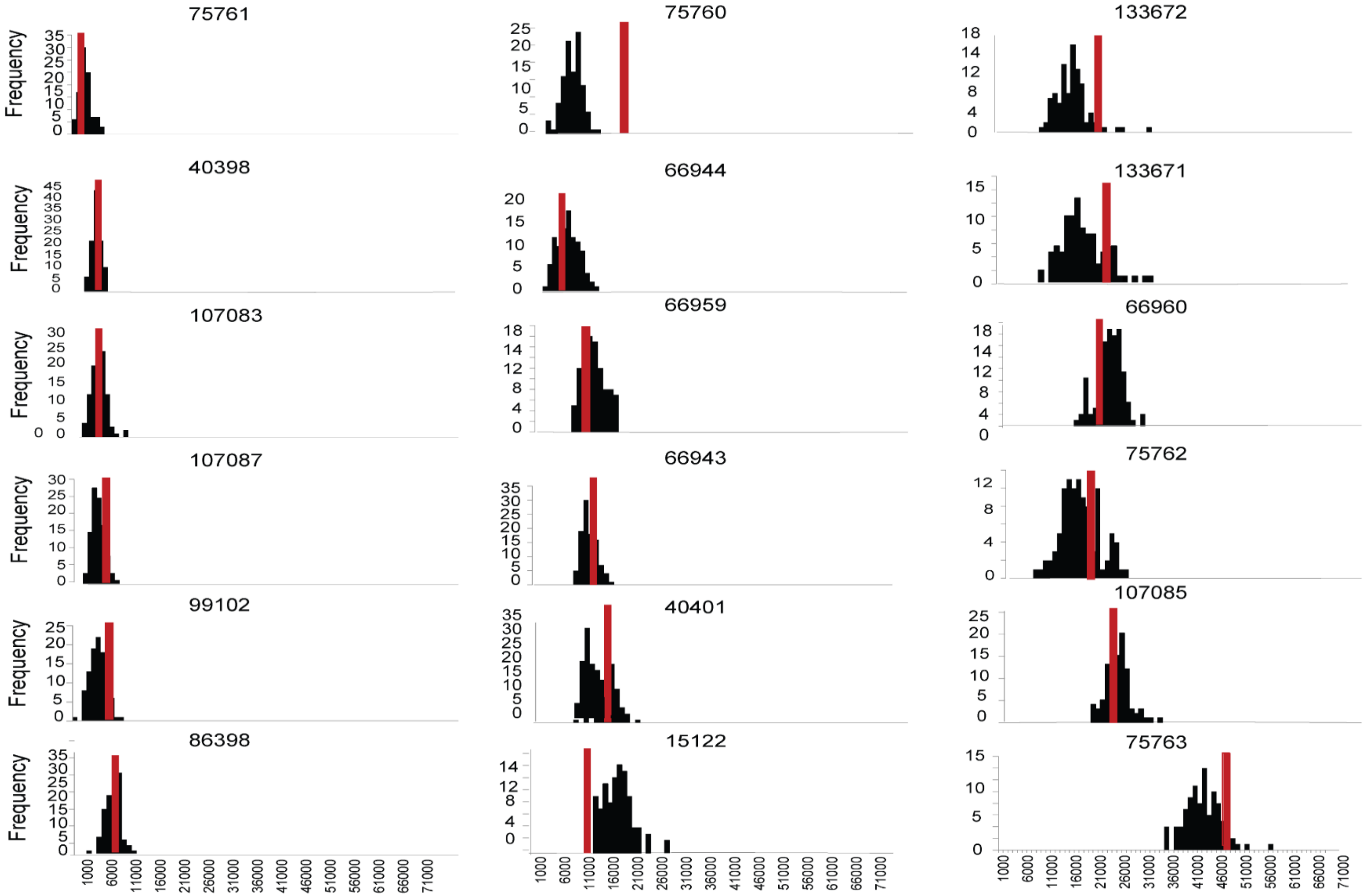

Figure S9 Individual sunfish real tracks (red) summed PD encountered in relation to the averaged random (black) plankton totals.

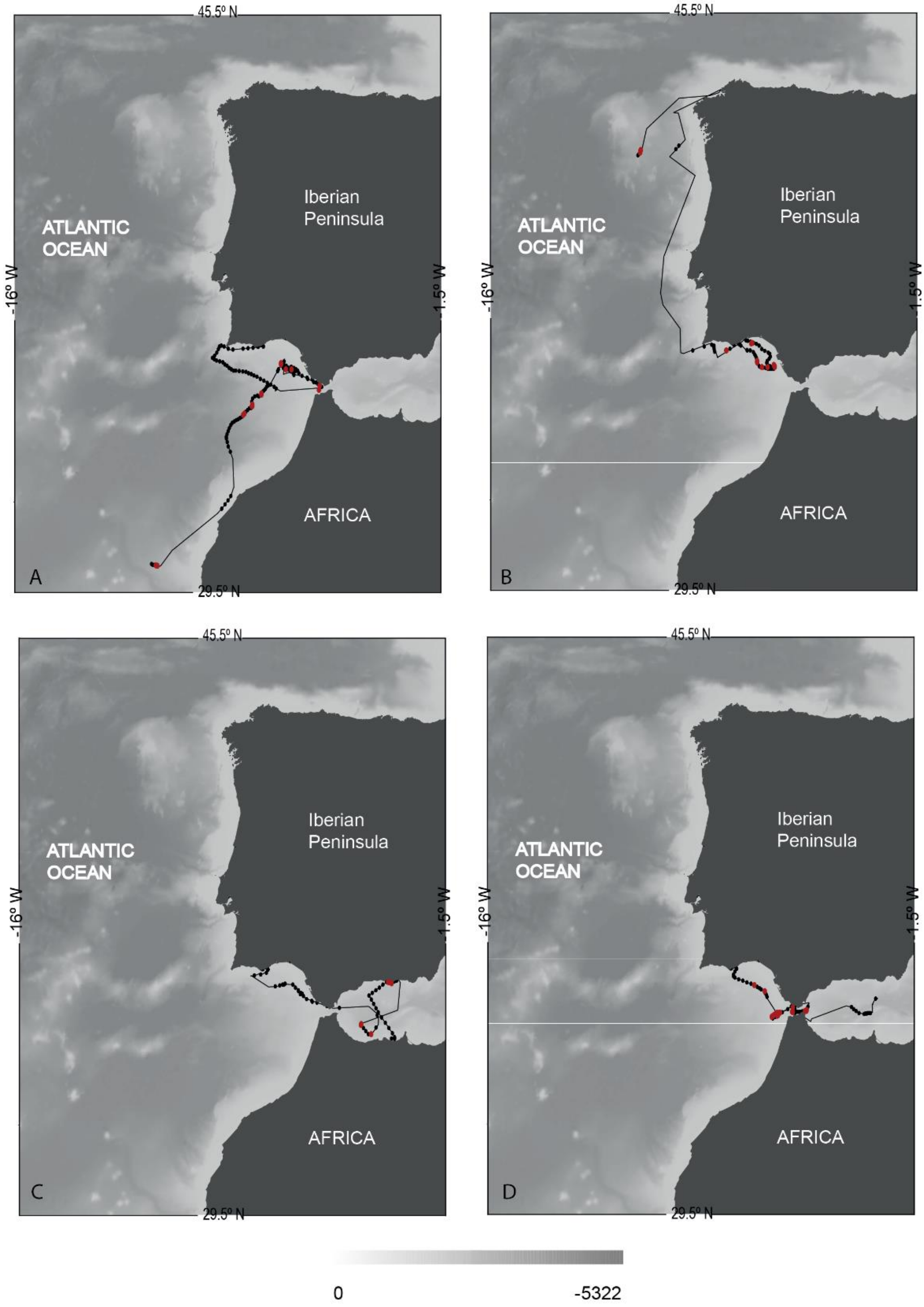

Figure S10 Individual sunfish GPS tracks (black) with ARS highlighted (red) overlaid on bathymetric map (m).

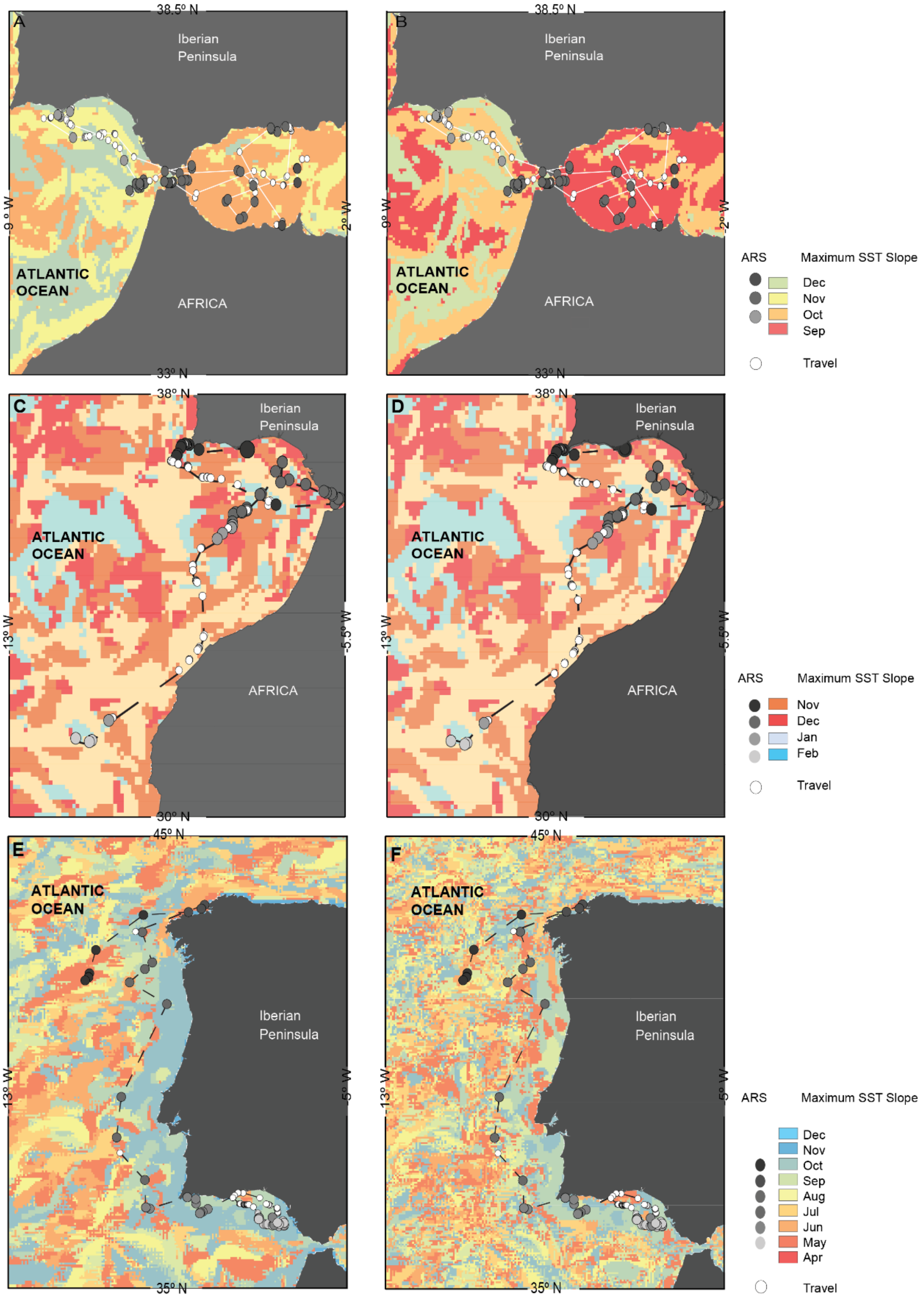

Figure S11 Sunfish tracks monthly coloured (grey scale when in ARS and white when travelling) superimposed on SST monthly maximum gradients maps. Tracks 2013 (#S17 and 18) with gradient of monthly maximum thermal gradients on the background (A), second highest SST slopes month (B); Track 2008 (#S8) on monthly maximum SST slopes (C), second highest month (D); Track 2010 (#S9) overlaid with gradient of monthly maximum thermal slopes (E), second highest productive month (F).

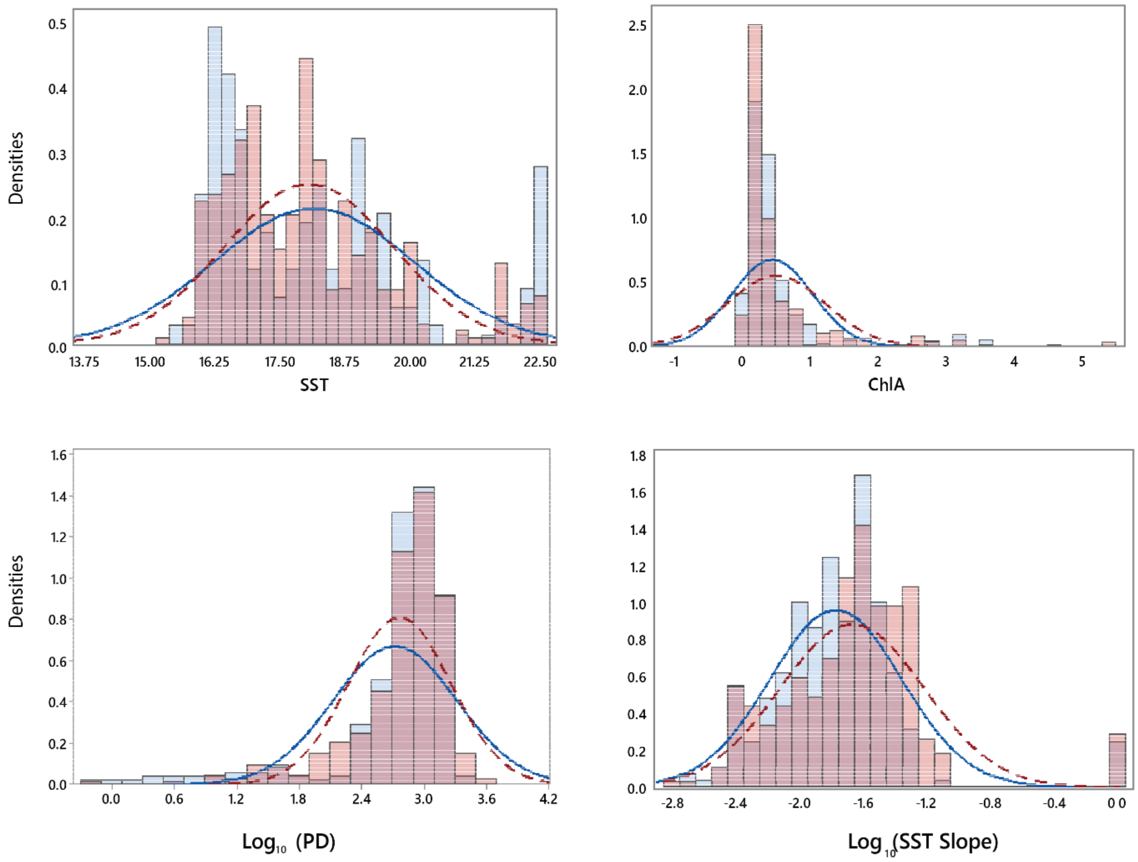

Figure S12 Histograms (overlapped not stacked) of encountered SST, chlorophyll *a*, plankton densities and SST gradients along sunfish tracks, denoting both behavioural modes (travel and ARS).

Table S 1 Full model coefficients for the probability of sunfish ARS occurrence as a function of plankton densities, SST, SST gradients and chlorophyll *a.*

Predictor	**edf**	**Ref.df**	**F**	***p-value***
Plankton density	1.000	1.000	8.467	0.00407
SST	5.880	5.880	6.161	0.00001
SST gradients	1.519	1.519	9.533	0.00067
chlorophyll *a*	1.000	1.000	3.088	0.08058

A.3 Chapter 5 : Supplementary methods

A.3.1 Denoising of sequences

A 454 platform standard flowgram output file per sunfish size class was obtained from Beckman Coulter Genomics® and after separating bacterial nucleotide sequences, the remaining bulk was filtered as follows:

1. Pyronoise procedure implemented in Mothur: a) **trim.seqs** function in mothur was used to remove the known mid tags barcode and primer sequences, while also rejecting all sequences with length smaller than 300 bp long and with homopolymers longer than 8 bp; b) **unique.seqs** was further implemented to simplify the dataset, by removing duplicates, leaving only the unique sequences; and lastly c) **chimera.uchime** was applied to the unique sequences screening for sequences formed from two or more biological sequences joined together (chimeras – thought to emrge from incomplete extension during PCR). Chimeric sequences were removed from the pool.

2. Filtering amino acid sequences in Macse: Nucleotid sequences retrieved from mothur procedure were then translated into protein sequences using Macse v1.01b (Ranwez, Harispe et al. 2011). Macse can detect interruptions in open reading frames, either caused by nucleotide substitutions that result in stop codons or the insertion or deletion/nucleotides that induce frameshifts (Leray, Yang et al. 2013).Thus, an automatic translation was performed while rejecting sequences with stop codons likely to belong to bacteria or pseudo genes and with frameshifts which presumably result from errors during the 454 platform sequencing procedure.

A.3.2 Taxonomic assignment of filtered sequences

GenBank (Benson 2003) is a comprehensive public database of DNA sequences, supporting bibliographic and biological annotation, built and distributed by the National Centre for Biotechnology Information (NCBI), USA. Each GenBank record consists of both a sequence and its annotations and is assigned a stable unique identifier, the accession number (Benson 2003).

BLAST is a search engine for sequence-similarity against a sequence database, such as GenBank, which works by locating regions of similarity between the sequence of interest and the database records. BLAST searches were performed on NCBI's web site[10] and received alignments had associated scores and measures of statistical significance (Wheeler 2003). Here, we used the GenBank database BLAST service to classify our filtered (a) nucleotide sequences, accepting the assigned taxon to those sequences with similarity higher than 98% (Altschul, Gish et al. 1990, Machida, Hashiguchi et al. 2009, Leray, Yang et al. 2013); (b) aminoacid translated sequences assigned to the lowest taxonomic group possible, given the homology attained per sequence.

For those sequences with BLAST similarity below the threshold of 98% we applied a Bayesian approach that allowed us to estimate the probability for the sample sequence to belong to a monophyletic group, identified with GenBank database sequences. Hence, we used the Statistical Assignment Package (SAP) (Munch, Boomsma et al. 2008), a Bayesian classifier, to classify each sequence to GenBank reference database by building 10,000 phylogenetic trees and calculating the posterior probability, via Markov Chain Monte Carlo (MCMC), that the query species belong to a particular taxonomic group, for all levels of taxonomic annotation. This posterior probability enables the assignment of the sequence to a higher ranking taxon, when homology information is too ambiguous at the species level (Munch, Boomsma et al. 2008). In summary, we permitted SAP to download 50 GenBank homologues at ≥70% sequence identity and accepted retrieved assignments only at the significance level of 95% (posterior probability) from the phylogenetic trees, at the respective lower taxon possible.

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[10] http://blast.ncbi.nlm.nih.gov/Blast.cgi

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